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NEW EVIDENCE IN FAVOR OF A CHIEF VASOCONSTRICTOR CENTER IN THE BRAIN

Studies in Vasomotor Reflex Arcs. IV

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From the Anatomical Laboratory of Northwestern University Medical School

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Our knowledge of the anatomy of the vasomotor reflex arcs is still very imperfect. We believe that there exists in the brain stem a center for the regulation of blood pressure; but it is debated whether or not there is a separate vasodilator center, (1) and whether there is in addition a separate vasotonic center (2). The location of these centers and their relation to each other is unknown. In the cat faradic stimulation of the fovea inferior in the floor of the fourth ventricle produces a sharp rise in blood pressure; and stimulation of a point 3 mm. behind this in the area postrema causes an equally marked drop in pressure (3). But no evidence has been presented to show that these points mark the location of a vasoconstrictor and a vasodilator center respectively. Possibly they are only the termini of afferent pressor and afferent depressor fibers of the vagus, i.e., only parts of the afferent limbs of the corresponding reflex arcs.

The afferent impulses producing the pressor reflex from the sciatic nerve are transmitted through the spinal cord in the apex of the posterior gray column (4). When this was cut on both sides of the cord at the level of the first lumbar segment no pressor reflex could be obtained from stimulation of the sciatic nerve. The apex of the columna posterior is composed of the substantia gelatinosa Rolandi and the tractus dorsolateralis of Lissauer. It contains no long tracts but only short association fibers linking one segment of the cord with another. Any impulse ascending or descending along this path for more than a segment or two must travel over a series of many neurones.

When we found that the afferent limb of the pressor arc included this series of short fibers we were led to surmise that this reflex might be largely spinal. According to this conception the impulses, enter-

ing the cord by way of the dorsal roots associated with the sciatic nerve (fig. 1, *b*) would ascend by way of these short spinal fibers in the apex of the posterior horn (*e*) to the thoracic and upper lumbar segments of the spinal cord and then forward (*g*) to the efferent vasoconstrictor neurones of the white rami (*a*). That such a spinal reflex is possible has been demonstrated by Pike (5) and Sherrington (6), who showed that after section of the cervical cord and during cerebral anaemia the vascular tone and pressor reflexes, which at first were lost, returned again after a short time. Obviously they must then have been purely spinal. The fact that these reflexes are temporarily abolished on section of the cervical cord does not prove that the corresponding arcs pass through the medulla, because all spinal reflexes are temporarily abolished under the same conditions. Either we must accept the hypothesis of Pike that in the mammal there are no spinal reflexes or we must admit that spinal shock (a disturbance of nervous equilibrium which von Monakow has called "diaschisis," Herrick (7)) may temporarily abolish the function of purely spinal arcs. If we make the latter admission it would apply as well to the vasomotor as to the other reflexes and we would be without evidence justifying us in locating the vasomotor reflex centers in the medulla. When analyzed in this way the conception of a chief vasoconstrictor center in the mnsdulla is seen to rest on rather insecure grounds.

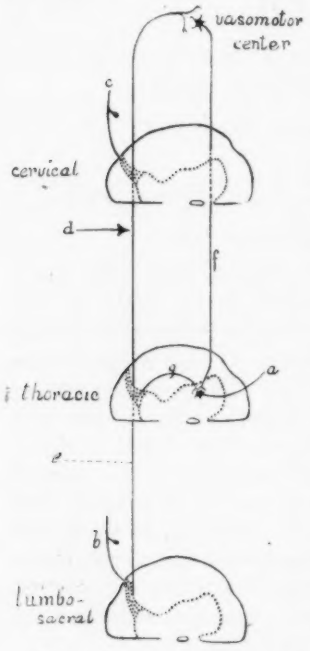

Fig. 1. Diagram to illustrate the paths which might be followed by vasomotor impulses: *a*, efferent fiber of a white ramus; *b* and *c*, afferent fibers of dorsal roots; *d*, level of a lesion in the posterior gray columns; *e*, line indicating the path of afferent pressor impulses in the posterior gray column.

Any experiment designed to test this conception must be so carried out that spinal shock or "diaschisis" is not produced. In other words the skeletal reflexes must not be affected by the conditions of the experiment. It is with evidence of this kind that the present paper deals. The apex of the posterior gray

column, including the dorsalateral tract of Lissauer and the substantia gelatinosa Rolandi, was cut on both sides of the spinal cord at the level of the first thoracic segment. This interrupts the pathway for afferent pressor impulses at the level of the arrow, *d*, figure 1. This is above the level of the thoracico-lumbar autonomic outflow through the white rami (*a*) but below the origin of the brachial nerves (*c*). If the pressor arc were purely spinal the sciatic reflex would not be affected by this lesion. The impulses from the sciatic entering along the unmyelinated fibers of the dorsal roots (*b*) would ascend in the apex of the posterior horn (*e*) to the thoracic and upper lumbar segments of the spinal cord and then pass forward through the gray matter (*g*) and out through the white rami (*a*). The lesion at *d* would however interrupt a hypothetical pressor path from the brachial nerves (*c*) down the apex of the posterior gray column to the thoracico-lumbar autonomic system (through *g* to *a*). In other words if the pressor reflex were purely spinal the lesion in question should not affect the reaction from the sciatic nerve but should abolish the reflex from the brachial nerves. But if the reflex were bulbar and the afferent limb of the arc followed the apex of the posterior column through the spinal cord to the medulla, the lesion would interrupt the arc from the sciatic nerve but not that from the brachial. This experiment gives us an opportunity to determine whether the pressor reflex is under normal conditions purely spinal or to what extent a bulbar chief vasoconstrictor center is involved.

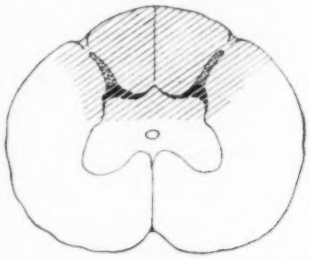

Fig. 2. Diagram showing the extent of the lesion in the second thoracic segment of the spinal cord of cat B10.

TECHNIQUE

Adult cats were used for these experiments. The spinal cord was exposed at the level of the second thoracic segment under rigid aseptic precautions. Since it had been previously shown that the posterior funiculi were not concerned in any of the vasomotor reactions an extensive lesion was made involving both posterior gray columns and both posterior funiculi (fig. 2). This operation is simpler and more certain than that of cutting the apices of the posterior columns

alone and answers the purpose of the present experiment just as well. The animals recovered promptly and showed no signs of paralysis. They were tested at intervals for sensibility to pain. The technique throughout was the same as in the earlier experiments from the account of which we quote (4):

> After complete recovery of the animal from the lesion and after the conduction of pain in the cord had been tested the vasomotor reflexes were studied. Under ether anaesthesia a tracheotomy was performed and the ether bottle attached. Connections were made as rapidly as possible for carotid blood pressure and respiratory tracings. Both sciatic nerves were exposed, ligated and cut distally to the ligature. The central end was laid bare for some distance above the ligature and could be handled by the attached thread. On the left side three brachial nerves, viz.: the median, ulnar, and internal cutaneous, were exposed, ligated together, and cut distally to the ligature and thereafter treated as a single nerve. Care was taken not to stretch the nerves at any time. When not being stimulated, they were kept covered with other tissues to prevent drying.
>
> To eliminate passive dilation of blood vessels in the areas supplied by the divided nerves the limbs were constricted with heavy cord placed proximal to the elbow and knee joints. Fluctuations in blood pressure from pressure on the abdomen by flexion of the limbs during stimulation of a nerve were prevented by securely tying the legs to the animal board.
>
> The stage of ether anaesthesia is of the greatest importance. The animal was kept relaxed, with regular respiration, with pupils half contracted and with a brisk corneal reflex. Any tendency toward cyanosis was prevented.
>
> Faradic stimulation of the central ends of the cut nerves was used to elicit the reflexes. Standard platinum electrodes were applied at least half an inch from the cut ends of the nerves, held suspended by the threads with which they had been ligated. The electrodes were moved slowly along the nerves during stimulation. The source of the current was a Stoelting inductorium No. 7090 through the primary of which passed a constant half ampere current.
>
> Curare was used in some of the experiments.
>
> After the reflex vasomotor and respiratory responses had been recorded the animal was killed. A stretch of cord about 7 mm. long containing the lesion was removed and prepared by the pyridine-silver method and cut into serial sections. The serial sections were then studied to determine the extent of the lesion.

After the operations the animals were allowed to live for periods varying from four to thirty-six days before the vasomotor reflexes were tested.

RESULTS

We will discuss our observations on pain and the depressor reflexes in these cats in a subsequent paper and confine our attention here to a comparison of the pressor reflexes obtained from the brachial and

the sciatic nerves. That it is reasonable to compare the results of stimulation of the brachial and sciatic nerves is shown by the fact that in normal cats stimulation of these nerves gives equivalent results. This statement is based not only on our own experience but also on that of Porter and Richardson (8), who found by a long series of experiments with stimuli of given intensity, applied to the sciatic and brachial nerves in cats and other animals, that the result did not depend in any way on which afferent spinal nerve was stimulated. According to Vincent and Cameron (9) all sensory spinal nerves "yield similar reflexes if sufficient care be taken to develop in all cases an equivalent stimulation of a roughly equal number of fibers." In our work we

TABLE

Vasomotor reactions obtained from cats in which the posterior funiculi and posterior gray columns had been divided at the level of the second thoracic segment from 4 to 35 days previously. Faradic stimulation, secondary coil at 5. Rise or fall indicated in millimeters of mercury

CAT NO.	LESION		RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
	Extent	Duration			
		days			
B7	Complete	4	8	5	32
B9	Complete	6	28	30	46
B10	Complete	16	0	0	28
B11	Complete	18	0	0	18
B12	Incomplete	15	18	10	26
B14	Complete	30	− 6	− 8	14
B15	Complete	36	−26	−14	28
B16	Complete	35	16	0	40

have used the three brachial nerves held together by a common ligature. These have a bulk roughly equal to that of the sciatic. We feel that much safer conclusions can be drawn by comparing the results of stimulation of the two in the same experiment than by a comparison of the results from the same nerve in normal and injured animals.

We have for comparison the results obtained under similar conditions from stimulation of the normal cat's sciatic. Table 1 of a previous paper (4) shows that strong stimulation of the sciatic in 14 normal cats gave a rise in blood pressure in all but 2. The position of the secondary coil varied from 4 to 6. The rise obtained varied from 8 to 48 mm. Hg.

In the present series of experiments a good pressor reflex was obtained in each case from strong faradic stimulation of the brachial nerves with the secondary coil at 5. The rise in blood pressure varied from 14 to 46 mm. as indicated in the table. Stimulation of the sciatic with the same strength of current gave no response or a slight fall in four of the eight experiments. In three other cases a slight pressor

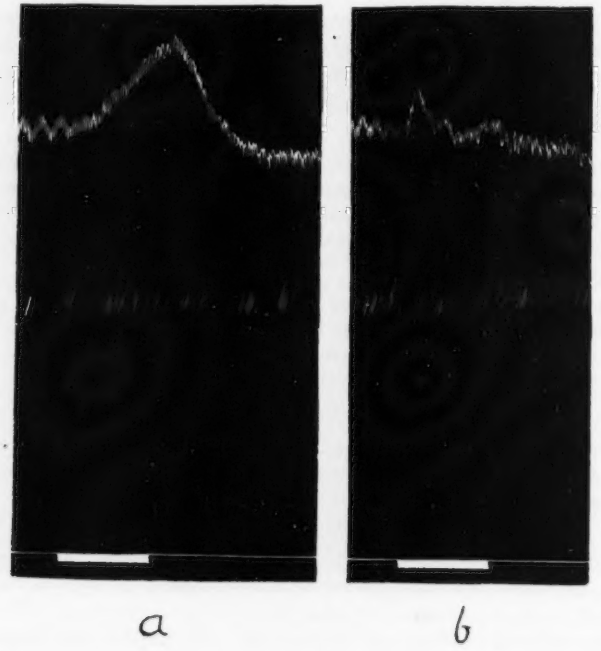

Fig. 3. Carotid blood pressure tracing from cat B10. Faradic stimulation, secondary coil at 5 of (*a*) three brachial nerves together and (*b*) the left sciatic nerve.

response was obtained from one or both sciatics. In one cat a good rise was obtained from both sciatics, but even in this case the reaction obtained from the brachial nerves was considerably greater.

Figure 3 shows the character of the reactions obtained from cat B 10, with cord lesion at the level of the second thoracic segment the extent of which is indicated in figure 2. The brachial nerves (*a*) gave a rise

in blood pressure of 28 mm. The left sciatic (*b*) gave no vasomotor reaction, but there were strong muscular contractions and these are indicated in the figure by the sharp irregularities of the tracing. The sciatic reaction was taken immediately after the brachial and the depth of anaesthesia and other conditions were the same. Such muscle spasms are frequently produced by stimulation of the sciatic in cats with lesions involving the posterior gray columns, when no spasms are produced by similar stimulation of the brachial nerves. We will discuss this observation in another paper.

Remembering that in normal cats the brachial and sciatic nerves give the same reactions, it is clear that in this series of experiments the lesion has more or less completely interrupted the pressor reflex arc from the sciatic without affecting that from the brachial nerves. This shows that the afferent pressor impulses from the sciatic travel up the cord to a vasoconstrictor center in the brain. That is to say the pressor reflex arc is not complete within the spinal cord. The path indicated by *g* in figure 1, carrying impulses from the apex of the posterior gray columns to the efferent vasomotor neurones, must have played a minor rôle, if any, in the reactions here recorded.

It is also obvious that the pathway from the vasoconstrictor center to the efferent vasomotor neurones must be located either in the ventral or lateral funiculi since it was injured in none of these experiments. This path is a part of the intact pressor reflex arc from the brachial nerves through the bulbar vasomotor center to the efferent vasomotor neurones of the white rami.

It is not possible at present to give a satisfactory interpretation of those cases in which more or less of a pressor reflex was obtained from the sciatic nerve. It may be that in these cases the pressor impulses passed forward along the path indicated by *g* in figure 1. We know that it is possible for the impulses to take this course after transection of the cervical cord. But before it could be said that this path functioned in these cases it would be necessary to be sure that the lesion had interrupted the only possible pathway for pressor impulses from the sciatic to the bulbar vasomotor center.

CONCLUSIONS

1. Pressor reflexes from the spinal nerves normally involve a bulbar vasoconstrictor center.

2. The afferent limb of the pressor arc from the sciatic nerve follows the apex of the posterior horn up to the level of the second thoracic segment and probably throughout the cord.

3. The efferent limb of the pressor arc lies in the lateral or ventral funiculi.

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AFFERENT SPINAL PATH FOR THE DEPRESSOR REFLEX

Studies in Vasomotor Reflex Arcs. V

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In the first paper (1) of this series it was demonstrated that the afferent impulses producing the pressor reflex travel along the cord in the apex of the posterior gray column. By a process of exclusion it was also shown that those responsible for the depressor reflex must be conducted by either the ventral or lateral funiculi. We were unable to secure a positive demonstration of this by the technique used in the former experiments because all those cats in which the ventral part of the lateral funiculi were cut developed paralysis and anaesthesia of the hind limbs and incontinence of urine. These symptoms did not appear in our other experiments and we are at a loss to account for them in these. However it seems not unlikely that hemorrhage from the ventrally placed extradural venous plexus may have resulted in a hematoma large enough to have produced pressure on the cord. In order to avoid this difficulty we abandoned the habit of letting the animals recover from the lesion before making the vasomotor tests, and carried out the operation and tests in one-half day.

TECHNIQUE

Under ether anaesthesia and without aseptic precautions the spinal cord was exposed in the region of the first lumbar segment. In working on the lateral funiculus it is necessary to prevent the knife from going through the dura into the extra dural venous plexus in the ventrolateral part of the canal. After the dura had been opened by a median longitudinal incision one tooth of the ligamentum denticulatum was cut and a strip of celluloid inserted between the cord and the dura in the interval between the fila of the first and second lumbar nerve roots. The knife was then passed through the lateral funiculus with

its sharp edge directed outwards and the cut was completed by pressing the edge against the celluloid plate. This procedure was then repeated on the opposite side. Two of the nine cats were allowed to come out from under the anaesthetic and their hind limbs were tested for sensibility to pain after which they were again anaesthetized and a record made of the vasomotor and respiratory responses to stimulation of the brachial and sciatic nerves. In the other eight cats the operation was done and the records obtained without interrupting the anaesthetic.

The experiment was followed by an autopsy and a microscopic study of serial sections of the portion of the cord containing the lesions. In this way the location and extent of the lesion was accurately determined.

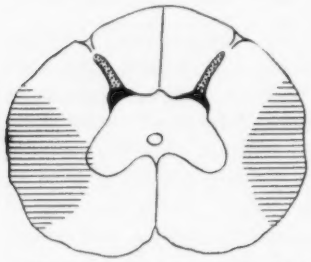

Fig. 1. Diagram of the first lumbar segment of the spinal cord of cat 91, showing the extent of the lesions in the lateral funiculi.

In testing the pain sense, pricking and pinching the skin and electrical stimulation through cutaneous electrodes were used as stimuli. A sharp cry and struggling were taken as evidences that pain was felt.

The technique of testing the vasomotor reflexes was the same as that given in the preceding paper on page 4 of this issue.

The autopsies showed that the lesion was in every case at the level of the first lumbar segment. The extent of the lesion as seen in microscopic sections varied considerably in the different cords but the lesions in cat 91, represented in figure 1, may be taken as typical. In some the injury was more extensive and in others less extensive than in this case.

RESULTS

When weak faradic stimulation was applied to the brachial nerves a drop in blood pressure was obtained in every case. As may be seen by reference to the table this drop varied in extent, the least being 5 and the greatest 32. These reactions represent typical depressor reflexes and the variation is no greater than in the reactions obtained from normal cats. They show that the vasomotor mechanism for depressor reflexes has not been impaired by the shock and hemorrhage incident to the preparation of the animal for experiment.

In sharp contrast to the reactions from the brachial nerves, are those from the sciatics. The same weak stimulation which consistently gave a drop when applied to the brachial nerves usually produced no effect when applied to the sciatics, and when there was a change in blood pressure it was as often a rise as a fall. The details are given in the table. The character of the reaction is shown in figure 2. It is evident that the lesions indicated in figure 1 interrupted the spinal afferent path for the depressor reflex from the sciatic nerves, figure 2, *b*.

But the lesions in the lateral funiculi did not interfere with the pressor reflex. On the contrary with faradic stimulation of medium intensity (secondary coil at 9 or 7) considerably better pressor reflexes were obtained from the sciatics than from the brachial nerves. Fig-

Table showing reflex changes in blood pressure after partial section of both lateral funiculi. Weak faradic stimulation, secondary coil at 15. Rise and fall indicated by + and − respectively and expressed in millimeters of mercury

CAT NO.	RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL
86	+4	+4	−32
90	−3	0	−10
91	0	0	−20
92	0	−2	− 8
93	0	0	− 8
94	−8	0	−16
99	0	0	−14
100	0	0	−10
101	+15	+10	− 5

ure 3 shows that faradic stimulation with secondary coil at 9 produced no response from the brachial nerves, but shortly afterward the same stimulation applied to the left sciatic nerve gave a considerable rise in blood pressure. With stronger stimuli (secondary coil at 5) there was no constant difference between the reactions obtained from the brachial and sciatic nerves.

These experiments seem to give additional evidence in favor of a balanced action between the pressor and depressor reflexes. In a former paper (1) the following theory was formulated:

> In the vasomotor reflexes there are two separate paths from the sciatic nerve to the efferent vasomotor neurones and for brevity we will speak of a pressor path and a depressor path according to the vascular responses produced by the impulses which they carry. The impulses traveling along the two paths are

mutually antagonistic and in normal animals vasomotor reactions represent a balance between them. The depressor path has a low threshold, and by it weak stimuli are able to reach the efferent vasomotor neurones. The pressor path has a high threshold since it is composed of the short fibers and many synapses of the apex of the posterior horn. With weak stimuli the impulses pass up the depressor path only, resulting in a drop in blood pressure. With stimuli of medium intensity they pass up both paths but the strong pressor overcome the weaker depressor impulses, resulting in increased blood pressure.

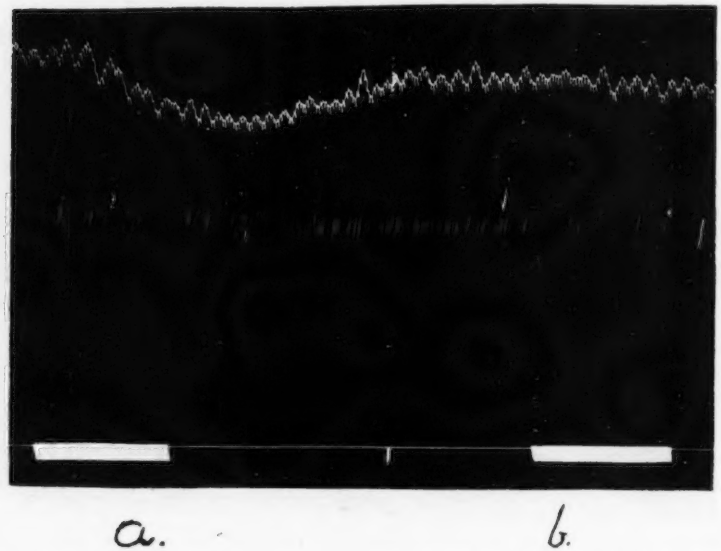

Fig. 2. Carotid blood pressure tracing from cat 91. Weak faradic stimulation (secondary coil at *15*) of the brachial nerves, *a*, and the left sciatic nerve, *b*. The drum was stopped at the point marked on the base line.

This theory is supported by the results of the present series of experiments. When the depressor path in the lateral funiculus has been divided, the pressor impulses arising from stimuli of medium intensity are able to manifest themselves in a rise in blood pressure, because they are no longer counterbalanced by the depressor impulses. In fact it would be difficult to account in any other way for the fact that, after section of the lateral funiculi in the region of the first lumbar segment, stimuli of medium intensity give better pressor reactions from the sciatic than from the brachial nerves.

There are two ways in which this balanced action might take place: 1. It may be a matter of inhibition, the depressor impulses inhibiting the vasoconstrictor center and the pressor impulses inhibiting the vasodilator center. 2. It may represent only the algebraic sum on blood pressure of the vasoconstriction and vasodilation resulting from the antagonistic afferent impulses.

Martin and Mendenhall (2) who have recently formulated a somewhat similar conception of the relation of the depressor to the pressor

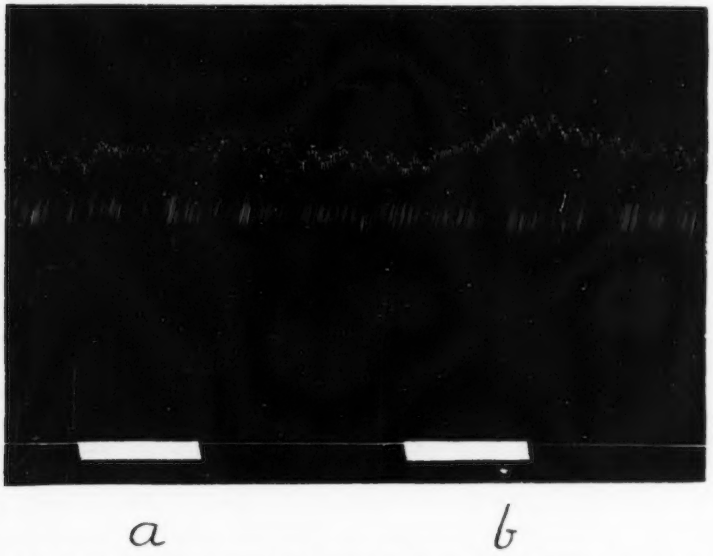

Fig. 3. Carotid blood pressure tracing from cat 91. Faradic stimulation of medium strength (secondary coil at *9*) of the brachial nerves, *a*, and the left sciatic nerve, *b*.

reflexes believe that the balance is in the form of the algebraic summation of the two actions rather than in the form of a mutual inhibition of the two centers.

The fact that after the section of the lateral funiculi at the level of the first lumbar segment the pressor reflexes from the sciatic are as good or better than those from the brachial, is important evidence in favor of the specific character of the results obtained by the division of the posterior gray columns in our other experiments. Against

all these other experiments the objection might well have been raised that there was perhaps nothing specific in the results. They might be explained on the assumption that strong impulses must reach the vasomotor center to produce a rise in blood pressure, and any extensive cord lesion involving afferent paths might so reduce the bulk of the impulses reaching this center as to weaken or obliterate the rise. We had shown, it is true, that the long fibers of the posterior funiculi had nothing to do with the vasomotor reactions. The present set of experiments completes the proof that the afferent impulses for the pressor and depressor reflexes follow separate paths in the spinal cord. The pressor path in the posterior gray columns can be interrupted without interfering with the depressor reflex; and the depressor path in the ventral funiculus can be interrupted without interfering with the pressor reflex.

A glance at figure 1 at once suggests the idea that the depressor path occupies much the same position in the cord as the spinothalamic tract. It occurred to us that the two might be identical, and in this case the afferent impulses would pass to the thalamus and then down to the vasomotor center or centers, and the reflex should be obliterated by decerebration. The depressor reflexes in decerebrate animals do not seem to have been thoroughly investigated by any one. In the earliest studies of these reflexes decerebration seems to have been one of the standard methods of procedure and, also, in at least one recent study, by Martin and Mendenhall (2), it was employed. This clearly indicates that the depressor reflexes are not obliterated by decerebration. This conclusion has been verified for us by Mr. W. N. Rowley of the Physiology Department. He performed decerebration on a number of dogs and found that the depressor reflexes were present and perhaps somewhat exaggerated. We wish to express our appreciation of his help in making this point perfectly clear.

On the basis of Mr. Rowley's work we may say with certainty that the depressor path is not the same as the spinothalamic tract although it occupies the same position in the spinal cord.

In the two cats which were allowed to come out from under the anaesthetic between the operation and the vasomotor tests no hypalgesia of the hind limbs could be demonstrated.

CONCLUSIONS

1. The afferent spinal path for depressor reflexes lies in the lateral funiculus in the same position as the spinothalamic tract, but it is composed of fibers that do not ascend above the rhombencephalon.

2. Section of this part of both lateral funiculi at the level of the first lumbar segment obliterates the depressor reflex from the sciatic nerves. The pressor reflex from stimuli of medium intensity applied to the sciatic nerves appears to be augmented.

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AFFERENT SPINAL PATHS AND THE VASOMOTOR REFLEXES

Studies in Vasomotor Reflex Arcs. VI

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There are many problems connected with the vasomotor reflexes which are still obscure. One of the most fundamental of these is the question: Why does the stimulation of a sensory nerve cause a rise in blood pressure under certain conditions and a drop under other conditions? It has long been known that stimulation of the depressor nerve gives only a drop in blood pressure, and that under the usual conditions stimulation of afferent spinal nerves gives only a rise. But, as was shown by Hunt (1) under carefully controlled conditions almost any afferent nerve can be made to give a depressor reflex. When a nerve is chilled the same stimulus, which before gave a rise, will cause a drop in pressure. It was known by Hunt and has been recently emphasized by Porter and others (2) that very weak stimuli will quite uniformally give depressor reflexes from any afferent nerve, except perhaps the splanchnic, and that strong stimulation of any afferent nerve, except the depressor nerve, will give a pressor reflex. It has been quite generally assumed that these facts could best be explained by the assumption that there are two kinds of afferent vasomotor fibers which have been called pressor and depressor fibers respectively. We propose to examine this old hypothesis in the light of our present knowledge of the afferent nervous system and of the vasomotor reflexes.

Very recently Vincent and Cameron (3) have opened up again the whole question of the significance of the depressor reflexes by some very suggestive observations: namely, that when an animal is under a certain light degree of ether anaesthesia, stimulation of afferent nerves produces very rapid deep respirations which in themselves produce a drop

in blood pressure. Opening the thorax or giving curare does away with this respiratory effect and converts the drop into a rise. They state that they have not been able to observe a fall in blood pressure from weak stimulation nor from the stimulation of a nerve that has been cooled when the thorax has previously been opened. This is calculated to throw doubt on the existence of a true depressor reflex from the spinal nerves. We shall refer to these observations again.

We have shown that there are in the spinal cord two separate paths to the vasomotor centers—one for pressor and the other for depressor impulses. It is the purpose of the present paper to present additional evidence along this line, to summarize that which has been presented in other papers of this series and to consider the relation of these observations to the general theory of the vasomotor reflexes.

The object of the experiments, which are to be reported here, was to determine whether the effect of a lesion in the pressor path was dependent on the level of the cord in which it was located. This investigation was suggested by the difference in the reflexes obtained from cats in which the pressor path had been destroyed at the level of the first lumbar segment (4) and those in which it had been destroyed at the level of the second thoracic (5). Accordingly three additional series of experiments were made in which the apices of the posterior horns were divided at the level of the third lumbar segment in six cats, at the level of the first lumbar segment in seven cats, and at the level of the ninth thoracic segment in four cats. In order to determine whether the conduction was homolateral or contralateral the apex of the posterior horn was divided only on the right side in the first lumbar segment of six other cats.

TECHNIQUE

The procedure was the same as that followed in the preceding investigations. The operations were aseptically performed according to the technique detailed in the first paper of this series (4). The cats recovered rapidly from the effects of the operation. They were allowed to live for varying periods and were with few exceptions in first class condition at the time of the experiment. One showed some slight paralysis of the hind limbs and two others were sick with an infectious disease of the respiratory tract. Repeatedly, at varying periods after the operation, the animals were tested for alterations of

the pain sense in the hind limbs. Finally a thorough examination was made of the vasomotor reflexes. The technique of testing the pain sense and making the tracing of the vasomotor reflexes was the same as that used in the preceding investigations. (4), (5). In each case an autopsy was performed, the lesion was accurately located and its extent determined by a study of serial sections.

Throughout these experiments the reactions obtained from the brachial nerves, situated above the lesion, were used as controls for the reactions from the sciatic. In another place (5) we have shown that in a normal animal identical reactions are obtained from the brachial and sciatic nerves, and that more reliable results can be obtained in this way than by comparing the sciatics of normal and injured animals.

BILATERAL LESIONS IN THE APICES OF THE POSTERIOR GRAY COLUMNS

The apices of both posterior gray columns were divided in twenty-five cats, in eight at the level of the second thoracic, in four at the level of the ninth thoracic, in seven at the level of the first lumbar, and in six at the level of the third lumbar segment. In five of these the vasomotor reactions were atypical since a rise in pressure was obtained from very weak stimulation. These atypical results are recorded by themselves in table 2. The reactions from the other twenty cats are given in table 1.

The first division of table 1 represents the vasomotor reactions obtained from cats in which the apices of both posterior gray columns had been divided along with the posterior funiculi at the level of the second thoracic segment. Note that with strong faradic stimulation (secondary coil at 5) good pressor reflexes were obtained from the brachial nerves in each cat, while from the sciatic nerves a moderate rise was obtained in three, no reaction in two, and a drop in two cats. It is obvious that the pressor reflex from the sciatic was decreased in every case, although in three cats it was not entirely obliterated. In presenting these results in another place (5), the question was raised as to whether the pressor reflexes obtained from the sciatics in these cats were purely spinal, i.e., whether there was in addition to the path by way of the medulla oblongata, also an alternative purely spinal arc for these reflexes which was complete below the second thoracic segment. We have introduced these results again

TABLE 1

Reflex changes in blood pressure after section of the apices of the posterior gray columns at different levels. Strong faradic stimulation, secondary coil at 5. Rise or fall in millimeters of mercury

CAT NO.	LESION			RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVE
	Level	Extent	Duration			
B7	II T.	Complete	4 days	+ 8	+ 5	+32
B9	II T.	Complete	6 days	+28	+30	+46
B10	II T.	Complete	16 days	0	0	+28
B11	II T.	Complete	18 days	0	0	+18
B14	II T.	Complete	30 days	− 6	− 8	+14
B15	II T.	Complete	36 days	−26	−14	+28
B16	II T.	Complete	35 days	+16	0	+40
80	IX T.	Incomplete	30 days	− 5	− 5	0
81	IX T.	Incomplete	29 days	+ 5	+12	+20
82	IX T.	Incomplete	26 days	−20	−16	+30
83	IX T.	Complete	26 days	−14	− 6	+16
B1	I L.	Complete	3 hours	−12	+10	+40
B2	I L.	Complete	1 day	−30	−34	−28
B4	I L.	Complete	24 days	0	0	+36
76	I L.	Complete	13 days	−26	−14	+44
77	I L.	Incomplete	12 days	−20	+20	+40
68	III L.	Complete	29 days	− 3	− 3	+12
69	III L.	Complete	30 days	−10	− 8	+20
71	III L.	Complete	30 days	−25	−30	+30
72	III L.	Complete	25 days	−30	−28	+28

TABLE 2

Atypical vasomotor reflexes obtained from cats in which very weak stimulation gave a rise in blood pressure. The apices of the posterior gray columns had been divided. Strong faradic stimulation, secondary coil at 5. Rise in millimeters of mercury

CAT NO.	LESION			RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
	Level	Extent	Duration			
B12	II T.	Incomplete	15 days	18	10	26
78	I L.	Complete	14 days	20	30	40
79	I L.	Incomplete	14 days	4	8	16
67	III L.	Complete	34 days	22	20	56
70	III L.	Complete	30 days	30	10	50

in this place in order to compare them with those obtained from cats with similar lesions at other levels.

The second division of table 1 gives the results obtained from cats in which the apices of both posterior horns had been divided at the level of the ninth thoracic segment. With the exception of one case, in which the lesion was incomplete, strong faradic stimulation (secondary coil at 5) gave a drop instead of a rise out of the sciatic nerves.

The third division of table 1 gives the results obtained from cats in which the apices of both posterior horns were divided at the level of the first lumbar segment. The lesions in cat 76 were typical and their position and extent is shown diagrammatically in figure 1. With strong faradic stimulation (secondary coil at 5) a drop was usually obtained from the sciatic nerves and a rise from the brachial nerves (see figure 2 and the table). In two of these cats a drop was obtained from the right sciatic and a rise from the left, but in one of the two the lesion was incomplete. The other cats gave either no reaction or a drop from strong stimulation of the sciatic.

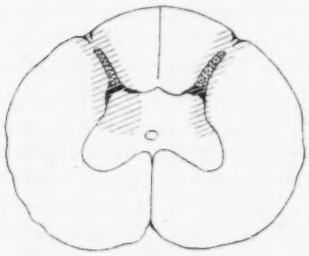

Fig. 1. Diagram of the first lumbar segment of cat 76 showing the extent of the lesions in the posterior gray columns.

The fourth division of table 1 gives the results obtained from cats in which the apices of the posterior horns had been divided at the level of the third lumbar segment. In each case strong stimulation gave a rise in blood pressure from the brachial and a drop from the sciatic nerves.

The last three divisions of the table show a pressor reflex only once from the sciatic when the lesion was complete and in this one cat the left sciatic only gave a rise while the right gave a drop. In one other cat with a complete lesion, B. 4, no vasomotor reflexes were obtained from the sciatics. In all the others with complete lesions strong stimulation of the sciatic gave a drop instead of a rise. Comparing these results with those indicated in the first division of this table it will be seen that there a rise is more frequent and a drop less frequent than in the last three divisions.

We believe that these differences are significant and that a lesion involving the posterior gray columns at the level of the second thoracic

segment produces less disturbance of the pressor reflex from the sciatic than lesions in this column lower down, because in the case of the higher lesion there is open an alternative path forward through the gray matter to the efferent vasomotor neurones. While impulses traveling this path are unable to develop the full pressor reflex as indicated by the reaction from the brachial nerves in the same animal, they are

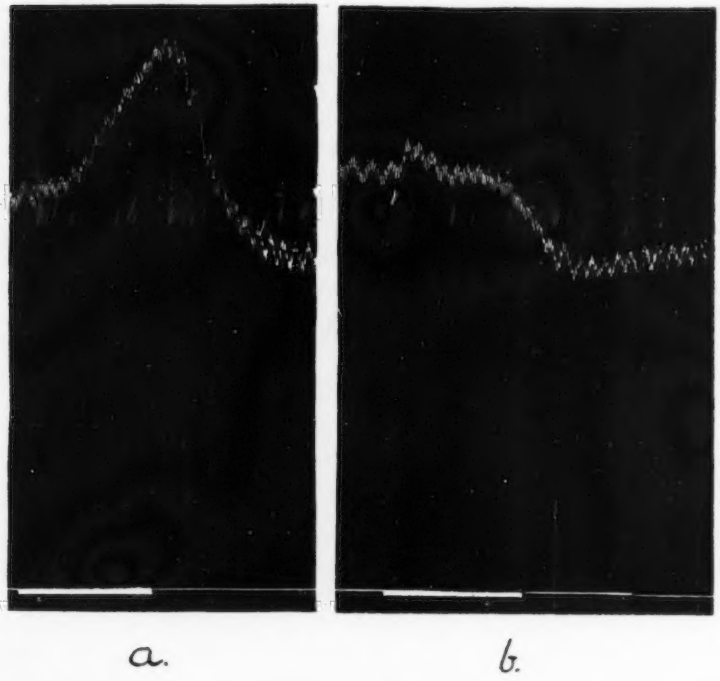

Fig. 2. Carotid blood pressure tracing from cat 76. Strong faradic stimulation, secondary coil at 5. *a*, brachial nerves; *b*, right sciatic nerve.

usually able either to produce a slight rise or to balance the depressor impulses, producing a negative result. This is what one should expect since it is known that such a purely spinal path exists after transection of the cervical cord (6).

The last three divisions of the table serve to emphasize in a striking

way the importance of the apices of the posterior gray columns for the conduction of the afferent pressor impulses in the spinal cord.

In five other cats, represented in table 2, the vasomotor reactions were atypical in that with very weak stimuli (secondary coil at 15) a rise in blood pressure was obtained. In all our other experiments stimuli of this strength have either given a fall or no change in pressure. In these five cats the lesion in the posterior gray column did not obliterate the pressor reflex from the sciatic nerves. It is obvious because of the weak stimuli with which they could be produced that some factor entered into these reactions, which is not present in the typical pressor reflex. Just what this factor is we are unable to say. Cannon and Hoskins (7) have shown that stimulation of the sciatic nerve causes the discharge into the blood of appreciable quantities of adrenin and this is probably not without effect on the blood pressure. The rise in pressure in our atypical cats occurred rather too promptly to be accounted for in this way. We bring up the matter of adrenin discharge not so much as an explanation as to show how diverse are the factors which enter into these reactions.

UNILATERAL LESION IN THE APEX OF THE RIGHT POSTERIOR GRAY COLUMN

It was shown in a former paper that after section of the right half of the spinal cord the pressor reflex from both sciatics was reduced, that from the right more so than that from the left. From these facts it was concluded "that the afferent impulses producing a rise in blood pressure are conducted bilaterally in the cord, and equally well on both sides or somewhat better homolaterally." In order to determine whether the pressor impulses travelled chiefly through the homolateral posterior gray column or equally in both, the apex of the right column was divided in six cats at the level of the first lumbar segment. The results are expressed in table 3. The animals were allowed to live from twelve to forty days before the vasomotor tests were made. The pressor reflexes obtained from the sciatics were less extensive than those from the brachial nerves and those from the right sciatic somewhat less extensive than those from the left. These results agree with those obtained from cats with laterally hemisected cords and show that the conduction of the afferent pressor impulses is bilateral but somewhat better on the homolateral side.

TABLE 3

Reflex changes in blood pressure after section of the apex of the right posterior gray column at the level of the first lumbar segment. Strong faradic stimulation, secondary coil at 5. Rise or fall in pressure expressed in millimeters of mercury

CAT NO.	RIGHT SCIATIC	LEFT SCIATIC	BRACHIAL NERVES
39	−20	+12	+20
40	+16	+18	+30
46	+ 8	+14	+16
47	+10	+20	+12
48	0	+10	+28
49	+14	+22	+28

THE PRESSOR ARC AND REFLEX

In this series of papers we have shown that the afferent impulses which cause a rise in blood pressure are initiated by strong stimuli. This is in keeping with the results of many other investigators. Martin and Lacey (11) have shown that the threshold for the pressor reflex is about 280 units as compared to 8.7 units for the depressor reflex, i.e., about thirty times higher for the former. In a few cases we have obtained a rise in pressure from very weak stimulation but in these it was evident that some unusual factor was at work. Not only was the stimulus too weak to give a true pressor reflex, but the rise was not obliterated from the sciatics by a lesion which did obliterate it in the other cats. So many factors enter into the determination of blood pressure that great care must be used in such a study as this to eliminate everything but the true vasomotor reflexes. We believe that the more successfully this is done the more evident it will become that the pressor reflex is elicited only by strong stimuli.

We have also shown that the afferent impulses, initiating the pressor reflex, are carried in the spinal nerves by the unmyelinated fibers (8). These are the fibers that mediate protopathic sensation. They enter the spinal cord along the lateral division of the dorsal root and run into the fasciculus dorso-lateralis of Lissauer in the apex of the posterior horn. In this they run for only a short distance before they end, probably in the substantia gelatinosa Rolandi. The apex of the posterior horn consists of Lissauer's fasciculus dorso-lateralis and the substantia-gelatinosa and receives the afferent pressor impulses as they enter the spinal cord.

The pressor pathway up the cord is composed of these same apical

structures. The tract of Lissauer consists not only of unmyelinated fibers from the dorsal roots but also of many fine endogenous fibers. These link one level of the substantia gelatinosa with another. In this way a chain of short neurones is established in the apex with the fibers in Lissauer's tract and the synapses in the substantia gelatinosa. There is reason for believing that this chain forms a path for the protopathic impulses involved in purely spinal reflexes; and it has been shown that this is the pathway up the cord for the afferent pressor impulses.

From table 1 of this paper we may conclude that these impulses ascend in the spinal cord to the thoracic segments, where a small percentage of them find their way forward through the gray matter (*g*, figure 1, page 2, of this issue) to the efferent vasomotor neurones of the white rami; but the greater volume of these impulses continues to ascend along this apical path to a vasoconstrictor center in the brain (5).

It has been said that this path consists of a series of short neurones linked together, The many synapses involved in this path offer a high resistance and are, we believe, the explanation of the high threshold for pressor stimuli. No other explanation has ever been offered for the fact that the threshold for these stimuli is thirty times greater than that for the stimuli adequate to produce the depressor reflex or the flexion reflex. In these numerous synapses we probably have the explanation of the latent period of the pressor reflex. That the latent period for the pressor reflex is greater than that for the depressor reflex, at least for stimuli of moderate intensity, has been very clearly demonstrated by Martin and Mendenhall (13). This is easily understood when it is known that the depressor path consists of long fibers and the pressor path of short fibers and many synapses.

The efferent path from the vasoconstrictor center to the efferent neurones of the white rami lies in the ventral or lateral funiculi but its exact position has not been determined. A diagram of this pressor reflex arc has been given in one of the preceding papers, figure 1 on page 2 of this issue.

We are not able to locate the vasomotor center. We know that there is located in the neighborhood of the facial colliculus a center which is responsible for the maintenance of blood pressure, the vasoconstrictor center of Dittmar (10). But when the floor of the fourth ventricle is explored with a needle electrode the only point which readily gives a rise in blood pressure is the fovea inferior. It may be that Dittmar's vasoconstrictor center is a vasotonic center and that a reflex vasoconstrictor

center is located in the region of the fovea inferior. But no convincing evidence has been presented to show that this is the case.

THE DEPRESSOR ARC AND REFLEX

The depressor reflex can be produced with great regularity by weak stimulation of afferent spinal nerves. Vincent and Cameron (3) seem to regard this as a somewhat unusual reaction. They say:

> We have not always found it possible to satisfy ourselves as to the different effects of weak and strong currents respectively by means of sliding the secondary coil up to or away from the primary, but we have on several occasions succeeded in demonstrating a marked qualitative difference in effect, using in the one case a single weak cell, in another one or more strong cells.

In our own work with cats we have found that if sufficient care be taken to have all conditions favorable weak stimuli give drops with great regularity and the strength at which the maximum drop is obtained is approximately the same for all cats. This is now so well established (2) that it is not necessary to present additional evidence.

Vincent and Cameron cast doubt on the vasomotor character of the depressor reaction obtained fron spinal nerves by showing that the excessive respiration which sometimes results from stimulation of a sensory nerve can produce a drop that closely simulates the depressor reflex. They deserve great credit for calling attention to this source of error, but it would be a mistake to ascribe all drops obtained from spinal nerves to this source. They say that they have never been able to notice the drop from weak stimulation of the spinal nerves when the thorax has been opened and the effect of excessive respiration has thus been eliminated. They call attention to the fact that curare, which stops respiratory movements, also eliminates the drop from weak stimulation of the spinal nerves.

All this might be taken to indicate that a true depressor reflex can not be obtained from the spinal nerves. But a study of respiratory and blood pressure tracings of a series of cats subjected to weak stimulation of the sciatic shows conclusively that the drop to be obtained from weak stimulation of the spinal nerves is independent of the respiratory movements. When the stage of anaesthesia is most favorable for the development of the depressor reflex the weak stimuli used often do not affect the respiration at all and quite as often inhibit as increase it. The drop in blood pressure is obtained as easily when the respiration remains unaffected or is inhibited as when it is slightly in-

creased. With stronger stimulation the greatly increased respiratory movements may no doubt play an important part in the drops in blood pressure which sometimes occur. Such respiratory effects play a part in the drops in blood pressure recorded in table 1 of this paper and table 6 of the first paper of the series (4). Wherever such strong stimuli are used that excessive respiratory movements are induced great care must taken in interpreting the results.

Weak stimulation of the vagus has the same effect as weak stimulation of the sciatic. Even greater drops in blood pressure can be obtained by moderately strong stimulation of the cat's vagus, to which very strong stimuli must be applied before a rise is produced. Only a fall in pressure can be obtained by stimulation of the depressor nerve. This difference in the afferent nerves has long been a matter of discussion and has been attributed to the existence of two kinds of afferent vasomotor fibers, with a different distribution in the peripheral nerves. We will now examine the evidence in favor of this time honored conception of special afferent pressor and depressor fibers.

a. Are there special afferent depressor fibers? Reid Hunt (1) is largely responsible for the prevalence of the conception that there are special afferent depressor fibers, since he assembled in good form the evidence available at that time. This in brief is as follows: Weak stimulation of most afferent nerves gives a drop in blood pressure. This can be obtained with stronger currents and with greater regularity if the nerves are cooled to about 5°C. At a certain stage in the regeneration of a nerve stimulation can be made to give a drop but not a rise in blood pressure. A drop is more easily obtained from the vagus than the spinal nerves and is always the result of stimulation of the depressor nerve. It was thought that these results might be most easily explained on the assumption that there were two kinds of afferent vasomotor fibers. If one made the additional assumption that the afferent pressor fibers are rendered nonconductive by cooling while the conductivity of the depressor fibers is unaffected or enhanced, and the further assumptions that the depressor fibers regenerate more rapidly than the pressor fibers, and that the depressor fibers are stimulated by weaker currents than the others, one would be in position to explain most of the facts then known. A weak stimulus applied to an afferent nerve would stimulate only the depressor fibers and give a drop in blood pressure, or if the nerve was cooled and the pressor fibers rendered non-conductive the impulses carried by the depressor fibers would cause a drop. At a certain stage in regeneration of a nerve only de-

pressor fibers would be present and their stimulation would cause a drop. One would only need to make the one additional assumption, that these depressor fibers are alone present in the depressor nerve, and predominate in the vagus and glossopharyngeal while the pressor fibers predominate in the spinal nerves, to be in position to explain the differences in the vasomotor reactions normally obtained from these nerves.

But if we examine the facts carefully we are not likely to take so much for granted. It is difficult to believe that two afferent fibers should be so differently constituted that the one would lose its conductivity by being cooled to 5°C. while the other would have its conductivity unimpaired or enhanced by the same temperature. So far as we know lowering the temperature decreases the conductivity of all nerve fibers. Cooling a nerve would decrease the strength of the impulses passing over it so that the net result would be the same as that of weak stimulation. The conductivity of a regenerating nerve is less than that of a normal one and only weak impulses could be made to reach the cord by way of such a nerve. It all reduces itself then to a matter of the strength and perhaps the volume of the afferent impulses which reach the spinal cord.

To our mind the most serious difficulty in the way of Hunt's explanation is to be found in the results of Martin and Lacey (11). According to them the threshold for the depressor reflex is 8.7 units as compared with 5.2 for the flexion reflex and 280 units for the pressor reflex. That is to say the depressor threshold is about the same as that of other reflexes while the pressor threshold is enormously higher. They say that stimuli 200 times above the depressor threshold are often used in experiments with the pressor reflex. It is difficult to conceive of two afferent fibers so differently constituted that the stimulation threshold of one should be 30 times greater than that of the other. Such differences in stimulation threshold may exist in end organs but not in nerve fibers.

As we have said all the facts so far as the spinal nerves are concerned are more easily explained on the basis of the strength and perhaps volume of the afferent impulses reaching the cord, and from our work on the dorsal roots we believe that it is only the protopathic afferent impulses that are here concerned. We still have to account for the segregation in the cord of afferent pressor and afferent depressor impulses and for the special reactions obtained from certain of the cranial nerves.

b. The afferent depressor path in the spinal cord. It has been shown (12) that the afferent impulses which produce the depressor reflex travel up the cord in the superficial portion of the lateral funiculus along a tract composed of long fibers with few relays. The resistance of this pathway is low as compared to that of the pressor path with its many synapses. Protopathic impulses on reaching the cord are received by the substantia gelatinosa from which two paths are open for them to the vasomotor centers: a path of low resistance in the lateral funiculus leading to the vasodilator center[1] and a path of high resistance in the apex of the posterior gray column leading to the vasoconstrictor center.

Weak impulses reaching the cord find their way along the former only and vasodilation (13) with a drop in blood pressure results. Whether they also inhibit the tonic action of the vasoconstrictor center is not so clear (14). With stimuli of moderate intensity some impulses force their way up the pressor path and produce vasoconstriction. Constriction and dilation balance each other with little change in pressure. There is reason for believing that the vasodilation is masked in this way rather than inhibited. But the failure of stimuli of a certain medium intensity to produce any change in blood pressure could be accounted for just as well by the assumption of a mutual inhibition. With strong stimuli, the resistance of the pressor path is overcome and the vasoconstrictor center brought into full action resulting in a rise in pressure. Here again it is not neccessary to assume the concomitant inhibition of the vasodilator center. The work of Martin and Mendenhall (13) would indicate that its action is only masked by the vasoconstriction, and table 1 of this paper shows that strong stimuli are very effective in producing a fall in blood pressure if the pressor path is interrupted. Vasoconstrictor fibers are much more widely distributed and their action is by its very nature more powerful than that of the vasodilators. When the vasoconstrictor apparatus is brought into full action by strong stimulation it is able to manifest itself in a rise of blood pressure irrespective of the action of the vasodilator center.

In brief we wish to explain the fact that weak stimulation of an afferent spinal nerve causes a drop while strong stimulation causes a rise in blood pressure on the basis of demonstrated spinal paths, the one

[1] The work of Martin and Mendenhall shows that active vasodilation occurs, and it is immaterial whether the center is anatomically distinct or is incorporated with the vasoconstrictor center. This Journal, 1915, xxxviii, 98.

with a low and the other with a high resistance. When one considers the synaptic resistance to be overcome in the pressor path, it is easy to understand why the threshold for pressor reflexes is 30 times higher than that for depressor reflexes. It is not easy to believe that one afferent nerve fiber has a stimulation threshold 30 times higher than another. It has been demonstrated that there are two such afferent spinal vasomotor paths, it has never been shown that there are two kinds of afferent vasomotor fibers.

c. Depressor reflexes from certain cranial nerves. The vagus and glossopharyngeal nerves give depressor reflexes more readily than spinal nerves. Our experience with cats shows that while weak stimulation of the vagus gives a drop, moderately strong stimulation gives a greater drop. A branch of the vagus, the depressor nerve, most easily studied in the rabbit, gives depressor reflexes exclusively. It has been supposed that the depressor nerve was composed exclusively of afferent depressor fibers and that the vagus and glossopharyngeal nerves contained relatively more of these fibers than the spinal nerves. Since the reactions from the fifth cranial nerve are the same as those from the spinal nerves, one would assume, according to this theory, that it had the same proportion of pressor and depressor fibers as they.

But there is another and better explanation for the special vasomotor reactions obtained from the vagus and glossopharyngeal nerves. These nerves are visceral nerves and their sensory fibers connect with the visceral afferent column in the medulla oblongata, that is with the fasciculus solitarius and its nucleus. The fifth cranial contains somatic afferent fibers as do the spinal nerves; and its sensory nuclei, the chief sensory nucleus and the nucleus of its spinal tract, form the upper end of the general somatic afferent column which extends through the spinal cord and medulla into the pons. The spinal tract of the fifth is the direct upward continuation of the tract of Lissauer, and its nucleus the direct upward continuation of the substantia gelatinosa Rolandi. With this somatic afferent column the vagus and glossopharyngeal nerves have only the very slightest connections. We have seen that the pressor pathway is intimately associated with this somatic afferent column and it is the somatic afferent nerves that most readily give pressor reflexes. The visceral nerves having only very slight connections with the somatic afferent column, give pressor reflexes with more difficulty.

As has been pointed out by Martin and Mendenhall (13) the high blood pressure produced by general vasoconstriction is designed to

supply the somatic musculature with sufficient blood during a period of muscular effort. We will quote this suggestive paragraph:

> It may not be amiss to point out that an important feature of the physiological significance of high blood pressure induced by vasoconstriction is in the diversion of much of the blood of the body into those organs which appear to have a vasodilator innervation but no constrictor: namely, the skeletal muscles.

Impulses from visceral nerves do not lead directly to activity of the skeletal muscles. It is the somatic afferent nerves which carry impulses initiating skeletal reflexes. Painful stimulation of the surface of the body initiates the movements necessary for escape or combat and at the same time brings about vasoconstriction and high blood pressure that the skeletal muscles may be better supplied with blood during the emergency. Reasoning in this way it is easy to understand why it is only the somatic nerves that have direct connections with the vasoconstrictor center. We believe that the known differences in the central connections of the visceral afferent and somatic afferent nerves is sufficient to account for the difference in the vasomotor reactions obtained from them without assuming that the one contains a larger number of special afferent depressor fibers than the other.

d. The vasodilator center. Weak stimulation of afferent nerves produces an active vasodilation (13). This implies the existence of a center for vasodilator reflexes. It is not known whether this center is anatomically distinct or incorporated within a general vasomotor center, Bayliss (14), Fananow and Tschalusson (14), and Martin and Stiles (15). We have shown that there exists in the floor of the fourth ventricle within the area postrema just lateral to the obex a "depressor point" direct faradic stimulation of which will cause a drop in blood pressure (9). Three millimeters higher up at the fovea inferior is the "pressor point" direct stimulation of which causes a rise in blood pressure. While the reactions to be obtained from these two points are highly specific we are not yet prepared to assert that they represent the location of the vasodilator and vasoconstrictor centers, although it is not improbable that such is the case.

e. The vasomotor balance. Many facts point to the conclusion that with weak stimulation the depressor apparatus alone is brought into play, while stronger stimulation gives a change in blood pressure which represents the balance between the action of the pressor and depressor mechanism. Martin and Mendenhall (13) are also of the opinion that pressor and depressor influences can be aroused simultaneously and

"the resultant effect on the peripheral vasomotor mechanism depends on the balance that is established between the opposing influences." Their results indicate that with the strong stimuli the action of the vasodilator center is masked rather than inhibited.

Our experiments have shown that when the depressor path in the cord has been divided in the upper lumbar region, stimuli of moderate strength will give a greater rise in blood pressure from the sciatic than from the brachial nerves (12). These results are most easily understood by assuming that with stimuli of moderate strength the change in blood pressure represents a balance between the action of the pressor and depressor impulses and that when the depressor impulses have been cut out the pressor impulses manifest themselves in a greater rise in blood pressure.

A glance at table 1 of this paper will show that when the pressor path has been interrupted in the lower thoracic or upper lumbar segments of the cord strong stimulation which gives a good pressor reflex from the brachial nerves will usually give a very considerable drop from the sciatic nerves. This would indicate that strong stimuli are at least as effective in exciting the depressor apparatus to activity as weak stimuli, but when the pressor mechanism is intact the net result is a rise.

In line with this reasoning is the fact that when one stimulates a spinal nerve with weak faradic currents an optimum strength can be found which will give the greatest drop. With increasing strengths the drop decreases in extent and finally gives place to a negative reaction, to a slight reaction which is variable in direction, or to a drop followed by a rise. With still stronger stimuli the pressor reflex appears and increases with the stimulus until a certain optimum strength is again reached. The point at which the reaction is negative or variable represents the strength at which the activity of the pressor balances that of the depressor apparatus.

THE AFFERENT PATH TO THE RESPIRATORY CENTER

We have not been able to locate any special part of the cord through which impulses ascend to the respiratory center. Bilateral section of the apices of the posterior horns interferes somewhat with the conduction of these impulses. In the same way and to about the same extent bilateral lesions in the lateral funiculi impede their conduction. These results indicate that the fibers which carry these impulses are widely scattered through the spinal cord and do not form a single compact fascicle.

THE CONDUCTION OF PAIN IN THE SPINAL CORD

The conditions under which painful afferent impulses are propagated along the spinal cord are not the same in the carnivora as in man. It is well established that in man pain impulses are carried by the lateral spino-thalamic tract (16). This is composed of long fibers which, beginning in the various segments of the cord, cross the median plane and extend uninterruptedly to the thalamus in the ventral part of the lateral funiculus. A lesion in this part of the cord in man produces total analgesia of the opposite side of the body below the lesion. In the cat, on the other hand, hemisection does not produce an appreciable degree of hypalgesia in either hind leg.

The conduction of pain in the cat's cord is bilateral and effected through a series of relays which give an opportunity for the impulses to enter the gray matter and cross the cord from side to side. This is clearly shown by the work of Karplus and Kreidl (17) who hemisected the spinal cord on the left side in the upper cervical and on the right side in the lower thoracic region. Cats, in which both sides of the cord had been thus divided and in which all long conduction paths to the brain had been cut, were still able to feel pain in the hind limbs. They conclude that pain is conducted in the cat's cord by a series of short fibers of the funiculus proprius system with frequent relays through the gray substance.

A study of the sensibility of the hind limbs in cats with various cord lesions confirms these conclusions. We could observe no hypalgesia after posterior hemisection (4), after lateral hemisection (4) or bilateral section of the lateral funiculi (12). We have repeated the double hemisection of Karplus and Kreidl on three cats and can confirm their results.

It would seem that the paths for pain conduction in the cat's cord are more diffuse than those for the conduction of the afferent vasomotor impulses. The latter are readily eliminated by appropriate lesions while nothing short of complete transection will stop the conduction of pain.

The most interesting observation in regard to the perception of pain was that after section of both posterior gray columns in the thoracic or upper lumbar regions the hind limbs were hyperaesthetic for a few days. The hyperaesthesia was noticeable shortly after the animal came out from under the anaesthetic and was usually at its height twenty-four hours after the operation. It gradually decreased and

could not be detected after a week or ten days. It is well known that an injury producing a hemisection of the spinal cord in man results in a more or less transient hyperaesthesia on the paralyzed side from the lesion down (18). It is probable that the hyperaesthesia observed by us in the cat is of the same sort and due to the same cause.

No satisfactory explanation has yet been given for the phenomenon in man. In our cats the hyperaesthesia was caused by the lesions in the posterior horns. We believe that all protopathic impulses are received in the cord through the tract of Lissauer and the substantia gelatinosa Rolandi (8), and that part of these impulses ascend in this apical complex to produce spinal and bulbar reflexes while part of them are transferred to special fiber tracts such as the pain path and the depressor pathway. It seems probable that when the pathway in the apex of the posterior horn is blocked a larger proportion of these impulses finds its way into the path for conscious pain. On the other hand it may be that the lesion increases the irritability of this apical system, through which the protopathic impulses pass on entering the cord. In favor of this latter explanation is the fact that the hyperaesthesia disappears in ten days or two weeks. Whatever the explanation, the fact remains that section of the apices of the posterior horns produces a transient hyperaesthesia of the body below the lesion. This fact is in line with our contention that the primary spinal mechanism for the reception of protopathic impulses is formed by the tract of Lissauer and the substantia gelatinosa which form the apex of the posterior horn.

A phenomenon, which is probably only another expression of this hyperaesthesia, has been mentioned in the fourth paper of this series (5). Figure 3 of that paper shows that, while stimulation of the brachial nerves gave a smooth pressor curve, stimulation of the sciatic gave an irregular curve the sharp rises in which were due to muscle spasm. In these cats with lesions in the apices of the posterior horn muscle spasms are much more likely to result from the stimulation of the sciatic than the brachial nerves. The muscle spasms are more marked if the experiment is made within the first few days after the operation at a time when the hyperaesthesia is evident, and we believe that the two phenomena have the same basis.

CONCLUSIONS

1. Afferent pressor impulses travel up the spinal cord in the apex of the posterior horn on both sides of the cord but somewhat better homolaterally.

2. It is probable that a small portion of the pressor impulses passes directly forward through the gray matter to the efferent vasomotor neurones of the white rami.

3. Most of the pressor impulses ascend through the apical path in the posterior horn to the vasoconstrictor center of the brain.

4. There is some evidence which points to the location of the reflex vasoconstrictor center in the region of the foeva inferior in the floor of the fourth ventricle.

5. The efferent path from the vasoconstrictor center to the vasomotor neurones of the white rami is located in the ventral or lateral funiculi, certainly not in the posterior.

6. The depressor path is located in the ventral part of the lateral funiculus and its conduction is bilateral although somewhat better on the contralateral side as indicated by the results of hemisection.

7. There is some evidence that a separate vasodilator center may exist in the region of the area postrema lateral to the obex in the floor of the fourth ventricle.

8. Weak stimulation of an afferent spinal nerve causes a drop in blood pressure which is a true vasomotor reflex and independent of respiratory movements.

9. A drop in blood pressure obtained from strong stimulation may be due to excessive respiratory movements.

10. True pressor reflexes are produced only by strong stimulation.

11. There is little evidence in favor of the time honored conception of two kinds of afferent vasomotor fibers and considerable evidence against it.

12. We have presented a theory of the vasomotor mechanism which accounts for the qualitative difference in the results of weak and strong stimuli on the basis of the resistance offered by the depressor and pressor paths respectively.

13. The glossopharyngeal and vagus nerves and the depressor branch of the latter are all visceral nerves and are connected with the visceral afferent column in the medulla while the fifth cranial and the spinal nerves are all connected with the somatic afferent column. This difference in the central connections of the two groups of nerves

is sufficient to account for the difference in the vasomotor reactions obtained from them.

14. No clearly defined path for afferent impulses to the respiratory center could be found. The fibers which carry these impulses seem to be somewhat widely scattered in the cord.

15. In the cat pain conduction in the spinal cord is bilateral and effected through a series of short relays.

16. For a few days following bilateral injury to the apices of the posterior gray columns there is hyperaesthesia of the hind limbs and all parts of the body behind the lesion.

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OSCILLATORY VARIATIONS IN THE CONTRACTIONS OF RHYTHMICALLY STIMULATED MUSCLE

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In 1904, Storey (1) reported that when electrodes are thrust into the gastrocnemius muscle of the cat and the muscle is stimulated by currents derived from a magneto-machine, the successive contractions rise and fall in "tonus rhythms." These rhythms, in Storey's experiments, began immediately after stimulation began, before fatigue occurred, and continued although the sciatic nerve was severed. A somewhat similar phenomenon was described by Symons (2), who noted that when certain frog, toad and mammalian muscles are fatigued with maximal break induction shocks temporary wave-like variations in the summit line can be produced in the later stages of fatigue by altering the load, the rate of stimulation, the temperature, or by introducing short periods of rest or of submaximal stimulation. Conditions inducing more rapid fatigue hasten the stage when the waves appear. Symons' observations on frog's muscle were confirmed by Burridge (3), who found the waves especially marked in fatigue curves obtained by perfusing the muscle with acid phosphates, carbon dioxide in Ringer's solution, and with lactic acid. Since the waves were not produced by perfusion with neutral salts (potassium chloride, sodium lactate, etc.) and since all the substances except ammonium lactate which produced the waves contain the hydrogen ion, the inferences were drawn that the hydrogen ion is a necessary concomitant of the waves, and that when they appear free acid is in process of neutralization by ammonia.

During recent experimentation on the effects of adrenalin on muscular fatigue we have subjected the tibialis anticus muscle, either by direct stimulation or by stimulation through its nerve, to rapidly and rhythmically repeated single stimuli, varying from about 35 to about 300 per minute. Both cats and dogs were used in the experiments, the former much more than the latter. The animals were anesthetized

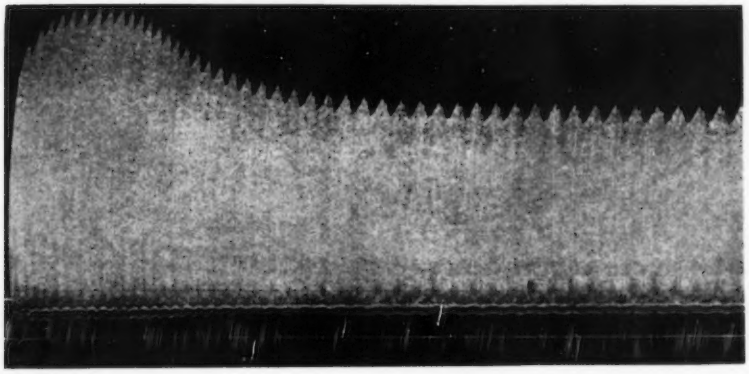

Fig. 1. Appearance of waves at beginning of contraction; waves longer as contraction continues. Record of tibialis anticus muscle stimulated 150 times per minute, and extending a spring having an initial tension of 120 grams. This record was taken from the beginning of the experiment and shows the "treppe" or initial improvement of the muscle. The waves persisted for about one hour and fifty-three minutes of continuous stimulation. Here and in other figures time is recorded in half-minute intervals.

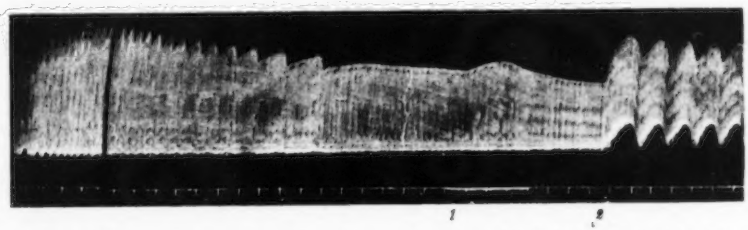

Fig. 2. Reappearance of waves when pull on muscle slightly increased. Record of tibialis anticus muscle, contracting 120 times a minute, and extending a spring having (as an after load) an initial tension of 100 grams. At *1* splanchnic stimulation began and continued for two minutes. At *2* the tension of the spring changed to pull continuously on the muscle (i.e., the muscle was "loaded").

with urethane (2 grams per kilo by stomach), or were studied without an anesthetic after being decerebrated under ether. No difference was noted between results obtained in these conditions. Only the tendon was exposed, through a slit in the skin; the contractile portion of the muscle was left *in situ*, supplied normally with its natural blood.[1] While at rest the muscle was slightly stretched, but otherwise not in stress; as soon as it began to contract it pulled against a spring having usually an initial tension of 100 grams. A characteristic feature of the muscular response under these circumstances was an oscillatory variation in the height of contraction (4). At present we are not able to offer any explanation of this phenomenon, but since we have noted a number of conditions which affect its appearance, disappearance, and alteration, an account of the phenomenon and some circumstances which influence it is now presented.

APPEARANCE AND DISAPPEARANCE OF WAVES

Conditions for the appearance of the oscillations. In our experience the oscillations have appeared most prominently in strong vigorous animals, and they have failed to appear only in a few animals that were sickly or asthenic at the time of observation. They begin to be manifested during the first contractions of the muscle while the initial improvement in the degree of shortening is still in progress, and therefore before fatigue, in the sense of lessened efficiency, has begun (see figs. 1 and 2).

As Cannon and Nice have already pointed out, if a fatigued muscle is shortening in a uniform manner, so that no oscillations are present, injection of a small dose of adrenalin or stimulation of a splanchnic nerve may promptly result in the development of rhythm (5). The renewal occurs although the arterial pressure in the muscle is kept at an even level.

Increase of tension may start waves again after they have ceased. In the instance represented in figure 2, the muscle at first was contracting 120 times a minute and pulling against a spring having (as an afterload) an initial tension of 100 grams. The waves ceased after about seven minutes. In the following six minutes, the height of contraction was increased as a result of splanchnic stimulation, but the

[1] For a detailed description of the method employed, see Cannon and Nice: This Journal, 1913, xxxii, 45; and Gruber: ibid., 221 and 438; ibid., 1914, xxxiii, 336; ibid., 1914, xxxiv, 89.

oscillations did not come back. Then the check for the spring was removed and its tension thus continuously applied to the muscle, and immediately the oscillations reappeared. There is apparently a limit to the amount of tension which can be applied to the muscle, favorable to the development of oscillations, for we have also noted that when they were present in an afterloaded muscle, they disappeared as soon as the muscle was loaded (see fig. 3).

Conditions for the disappearance of the oscillations. After the oscillations have begun they usually continue for a considerable period after the fairly even height of contraction due to fatigue has been reached (see fig. 1). The period during which they continue thus before being gradually obliterated varies in different animals. In the animal from which figure 1 was taken the waves were present during continuous stimulation for one hour and fifty-three minutes. In other animals the waves have been present as long as five hours. The impression which numerous observations have made upon us is that the persistence depends directly on the vigor of the animal. In several animals weakened by fasting we have seen the waves begin as usual with the first stimulations and quickly vanish before the fatigue level had been reached. In one cat, without food six days, nine oscillations were recorded, whereupon the muscle began to contract to a smooth and even line; and in another animal, without food four days, only two oscillations occurred, lasting one minute (see fig. 4).

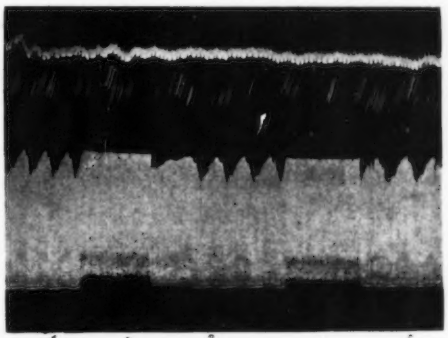

Fig. 3. Disappearance of waves with increase of pull on muscle. Upper record, blood pressure with mercury manometer. Lower record, the tibialis anticus muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams. At *1*, *3* and *5* the muscle was after-loaded, at *2* and *4* the waves disappeared as the muscle was directly loaded.

A strong dose of adrenalin (1 cc., 1: 10,000, intravenously), sufficient to cause a marked constriction of the arterioles, results in lessened height of contraction and slowing of the waves, and, if this decreased

Fig. 4. Influence of general condition on muscle waves. Upper record, blood pressure. Lower record, contraction of the tibialis anticus muscle (228 times a minute, extending a spring with an initial tension of 120 grams) in an animal fasted four days.

blood supply persists for a long time, in a banishment of the oscillations; but within a few minutes they start again (see fig. 5). This observation indicates that a sufficient supply of blood is a necessary condition for the existence of the waves, for in this case, although muscular contractions continued in spite of defective blood supply, the oscillatory variations in contractions did not. Nevertheless we have seen the waves in cases of low arterial pressure after the brain and upper spinal cord had been pithed, and also in excised perfused muscles.

The suggested relation of the oscillations to the arterial blood supply might indicate that the ups and downs of arterial pressure, especially after injection of adrenalin, would account for the variations in height of the muscle record (6). We have observed the rhythmic variations in muscular contraction, however, without any corresponding changes in the arterial pressure curve (see figs. 3, 4 and 6), and also we have records of rhythmic alterations in arterial pressure without corresponding waves in muscular activity (see fig. 5).

Fig. 5. Influence of diminished blood supply on muscle waves. Upper record, blood pressure. Lower record, the tibialis anticus muscle contracting 300 times a minute. At *1* adrenalin (1 cc., 1: 10,000, injected intravenously) abolished the waves but not the contractions. The waves gradually returned to their former rate.

Multiple oscillations. The records obtained from muscles, to which stimuli of uniform strength were applied at a uniform rate, were not always simple oscillations as observed in figure 1. Double and modified waves are frequently observed (see figs. 6 and 8). In figure 6 seven small waves are superimposed upon each large wave, which lasted about one minute. Some muscles recorded a

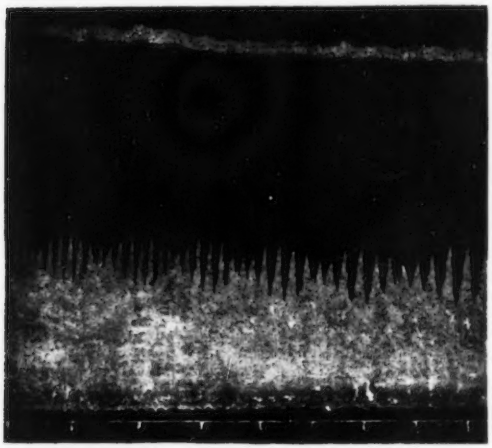

Fig. 6. Compound muscle waves. Upper record, blood pressure. Lower record, the tibialis anticus muscle contracting 240 times a minute, and extending a spring with an initial tension of 120 grams.

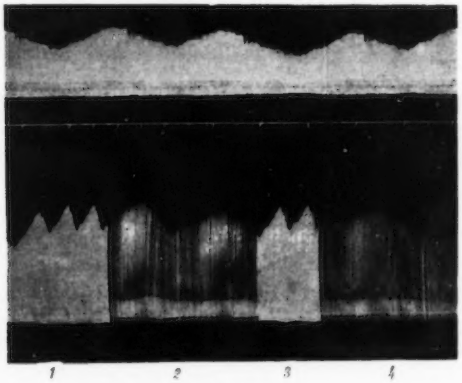

Fig. 7. Influence of rate of stimulation and of tension on duration of muscle waves. Lower record, muscle contracting, at *1* and *3*, 130 times a minute, and extending a spring with an initial tension of 100 grams. At *2* and *4* the stimulation rate was lowered to 40 per minute.

Upper record, same conditions as at *1* and *3* in lower record, except that initial tension of spring is 180 grams.

rhythmic alternation of a high and a low wave; and others contracted rhythmically in a "treppe" like manner in three or four wave groups each followed by a deep notch. Splanchnic stimulation, with the adrenal gland intact, sometimes changed these modified rhythms into the usual even and regular rhythm.

CONDITIONS AFFECTING THE OSCILLATORY RATE

Conditions causing an increase of rate. The waves are always faster at the beginning of the stimulation, when the muscular contractions are high and the initial improvement is in progress. At this point of maximal efficiency of the muscle, they occur at the rate of 2 to 20 per minute; they gradually decrease in rate as fatigue approaches and the height of the muscular contraction becomes low, until they finally disappear.

Fig. 8. Influence of small doses of adrenalin on rate and height of muscle waves. Record of the tibialis anticus muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams. At *1* and again at *2* adrenalin (0.3 cc., 1: 100,000) was injected intravenously.

Symons (2) observed that if the rate of stimulation is increased the wave-time decreases, i.e., the waves recur more rapidly. These observations are corroborated by our experiments. If the muscle is not too fatigued and the rate of stimulation is increased the waves appear at a much more rapid rate. This increase in rate has an optimal point beyond which the muscle rapidly fatigues to a smooth even curve. The effect of changing the rate of stimulation upon the waves can be seen by reference to figure 7. The initial tension was 100 grams. When the rate of stimulation was 130 per minute (at *1* and *3*), one wave lasted for about thirty seconds; but when the rate was reduced to 40 per minute (at *2*), a single wave lasted about ninety seconds, i.e., when the rate of stimulation was decreased from 130 to 40 per minute the duration of a single wave was correspondingly increased, lasting three times as long as before.

The tension against which the muscle contracts alters the rate of the

waves. With a constant rate of stimulation a decrease of tension increases the rate of the waves and an increase of tension decreases the rate. In figure 7 the rate of stimulation is 130 per minute in regions *1* and *3* in the lower curve and throughout the upper curve. The tension at the designated regions in the lower curve is about 100 grams and there result about two waves to the minute. In the upper curve the tension is 180 grams and one wave lasts about two and a half minutes.

Our results do not substantiate Symons' inference (7) that adrenalin has no effect upon the wave-like variations. We have observed that an injection of a small dose of adrenalin (0.1 to 1.5 cc., 1: 100,000, intravenously) or splanchnic stimulation or increased blood pressure may not only reëstablish the waves after they have disappeared (see p. 38), but increase the rate of the oscillatory rhythm if already present (8). In figure 8 two injections of adrenalin (0.3 cc., 1: 100,000, intravenously) were made. After the first injection (at *1*) there resulted a gradual increase in the wave rate. These waves, however, did not reach their maximal rate until after the second injection of adrenalin (at *2*). Upon further injection of adrenalin no additional increase was observed.

In some muscles when the tension was changed from an after load to a direct load the waves increased in rate and *vice versa*. This however is not always the case (see fig. 3).

Conditions causing a decrease of rate. The most prominent factor in bringing about a slowing in the wave rate is the oncome of fatigue. Under uniform stimulation the waves gradually increase in length, until the waves may last four or five minutes each and then finally disappear. Although Symons attributed these waves to fatigue, he noticed that fatigue also increases their duration. If the rate of stimulation is made slower, or the tension is increased with a uniform and constant rate of stimulation, the waves are prolonged (see fig. 7). Long waves were observed at the start in an animal that had been fasting (see fig. 4), and in normal animals after the blood supply had temporarily been shut off, and after a large dose of adrenalin had been injected intravenously (see fig. 5). In many animals these two latter conditions brought about complete obliteration of the waves.

OSCILLATIONS IN ISOLATED, DENERVATED AND CURARIZED MUSCLES

Not only were the waves observed in normal muscles (*in situ*), but also in muscles excised and perfused with Ringer's solution at 38.5°C. and at a pressure of 95 mm. of mercury (see fig. 9). This substanti-

ates the earlier statement that the waves are not dependent upon rhythmic alterations in arterial pressure but are the result of some property or properties of the muscle tissue itself. Since the waves are seen in denervated muscles either after nerve degeneration (9) (nerves cut nine to fifteen days before the experiment), or after paralysis of the nerve endings by curare it is evident that changes in irritability or conductivity of the nerve-trunks or nerve-endings supplying the muscle do not govern their appearance.

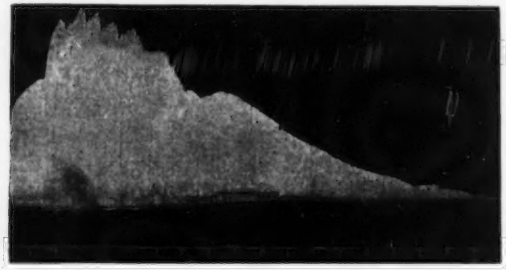

Fig. 9. Record of excised, perfused muscle contracting 120 times a minute and extending a spring with an initial tension of 120 grams.

SUMMARY

Wave-like variations in the height of contraction are obtained from mammalian muscles under rhythmical and uniform stimulation. These waves are similar to the waves observed by Symons and Burridge in their experiments upon frogs. In our experiments they occur too soon to be the result of a fatigue process.

The waves are related to the condition of the animal. In healthy vigorous animals they may be present for more than four hours; in sickly or fasting animals usually no waves or only a few occur.

Increasing the initial tension against which the muscle is contracting may occasion the appearance or the disappearance of the waves.

Injection of a small amount of adrenin or splanchnic stimulation may bring about a return of the waves after they have disappeared.

Fatigue or diminished blood supply (e.g., a dose of adrenin strong enough to constrict arterioles, or low arterial pressure) bring about a slowing or an entire disappearance of the waves. In healthy vigorous animals the waves usually return in a short time after the injection of a strong dose of adrenin.

Splanchnic stimulation, injections of a weak dose of adrenin (0.1 to 1.5 cc., 1: 100,000, intravenously), or a decrease in tension, each may increase the rate of the oscillations.

The muscle waves are observed in curarized or isolated or denervated muscles; in the body they do not coincide with waves of arterial pressure. They are, therefore, strictly of muscular origin.

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(5) CANNON AND NICE: Loc. cit., 54 (fig. 6).
(6) GRUBER: Loc. cit., 348 and 351.
(7) SYMONS: Loc. cit., 398.
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FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

IX. The Effect of Adrenin on the Factors of Coagulation

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It was noted in 1903 (1) that adrenin shortens the coagulation time of blood. The careful studies of Cannon and Gray (2) have established this action, have revealed in part its mechanism, and have explained the reason for previous discordant results. They have shown that minimal doses of adrenin constantly decrease coagulation time and that apparently the liver (and intestines?) is an essential to this action of the drug. They found that in large doses adrenin increases coagulation time; and Howell (3) has described a condition akin to hemophilia which he was able to produce in dogs by the injection of massive doses. From these observations it seemed highly probable that a study of the effect of adrenin on the factors of coagulation would throw some light on the source of these factors in the animal body.

The experimental procedure was as follows: The cats were etherized and a tracheal cannula inserted. Under deep ether anaesthesia they were pithed to the midthorax through the orbit. Artificial respiration was immediately begun. The right femoral artery was then bared and a vaselined cannula inserted from which the specimens for analysis were drawn. The left femoral vein was also fitted with a cannula into which the injections of adrenin[1] were made. After about forty minutes had elasped (to allow the animal to recover from the immediate "shock" of pithing) the coagulation time was determined. The method employed was that of drawing about 1 cc. of blood into a small test tube and inverting the tube cautiously every fifteen or thirty seconds. As soon as this determination was made the first specimen of blood for analysis was drawn. All such specimens were drawn directly into vaselined centrifuge tubes containing a 0.1 per cent

[1] The adrenin used was that prepared and sold by Parke, Davis & Co., under the name of "Adrenalin Chloride."

solution of sodium oxalate in Ringer's solution. There was enough oxalate in the tube to make a solution of 0.1 per cent of oxalate in the blood. The tubes and cannulae were vaselined by immersing them in a saturated solution of petrolatum in ether, allowing them to drain and the ether to evaporate. Sometimes the specimens were centrifuged as soon as they were drawn and sometimes allowed to stand until the end of the experiment.

After the plasma was separated by means of centrifugalization at high speed for twenty minutes, prothrombin time and antithrombin were determined by the methods of Howell (3). This procedure is in brief as follows: To 5 drops of oxalated plasma in small test tubes are added respectively 2, 3, 4, 5 and 6 drops of a 0.5 per cent $CaCl_2$ solution. The tubes are inverted every fifteen seconds and the time at which a firm clot appears is called the "prothrombin time." The first tube to clot is the one containing the optimum amount of calcium chloride and it is this time which is used as the index of the prothrombin present in the specimen.

The plasma remaining from the prothrombin determination is heated to 60°C., precipitating the proteins, is then centrifuged, and the clear supernatant fluid drawn off and used as an antithrombin solution. Varying amounts of a solution of thrombin, prepared from beef fibrin (4), were placed in small test tubes and one drop of the antithrombin solution added. This mixture was allowed to stand for fifteen minutes and then 10 drops of a solution of "dried oxalated plasma" (4) added. Five tubes were usually used. The time necessary to produce a firm clot in each tube was noted. Either the average of all five times or the average of the three middle tubes was used as an index of the amount of antithrombin present in the specimen. This determination was controlled by setting up a similar series of tubes without antithrombin. Such tubes invariably clotted in from five to ten minutes.

The fibrinogen determinations were made by the heat coagulation method of Whipple and Hurwitz (5) an unsatisfactory and unreliable method for this work, because, chiefly, the amounts of blood required for accurate results are too large to obtain from cats without introducing the factor of hemorrhage.

The "prothrombin time" (6), while in itself merely an indication of the coagulation time, can here be used as an index of the amount of prothrombin present because antithrombin in these experiments did not vary. In the first experiments the coagulation time and prothrombin time varied so much, due probably to the excitement incident to

confining and etherizing the animal, that it was decided, in order to eliminate such disturbance, to cut the splanchnic nerves before beginning the experiment. Under aseptic precautions an incision was made in the anterior abdominal wall and the nerves cut where they cross the crura of the diaphragm. Both the major and minor splanchnics were severed when both were found. The latter was not found at operation in about half the animals; neither nerve was ever found intact at necropsy. The operated animals were never used for experiments until the abdominal wound was completely healed—an interval of from two to eight weeks.

The following illustrative protocols will show the course of procedure and the details of the results:

Experiment VIII. July 2, 1915. Cat 15. Weight 3 k. Both splanchnic nerves cut June 15, 1915.

9.50 a.m. Etherized.
9.58 a.m. Pithed.
10.37 a.m. C. T.—10′—10′30″.
10.47 a.m. Specimen no. 1—4.5 cc. from right femoral artery.
10.49 a.m. C. T.—7′.
10.55 a.m. 0.4 cc. *adrenin* (1: 100,000) into left femoral vein.
10.59 a.m. C. T.—3′30″—4′15″.
11.04 a.m. Specimen no. 2—4.5 cc.
11.06 a.m. C. T.—2′15″.
11.25 a.m. C. T.—3′.
11.29 a.m. C. T.—4′45″—5′30″.
11.36 a.m. Specimen no. 3—4.5 cc.
12.17 p.m. C. T.—7′—7′30″.
12.26 p.m. Specimen no. 4—4.5 cc.
12.27 p.m. C. T.—3′30″.
12.31 p.m. C. T.—5′30″—6′30″.

Autopsy shows both splanchnic nerves divided.

Prothrombin.

									Optimum	*Check*
5 gtts.	plasma	no. 1	+2	gtts.	$CaCl_2$	Clotting time		4′		
"	"	"	3	"	"	"	"	5′		
"	"	"	4	"	"	"	"	5′		
"	"	"	5	"	"	"	"	5′30″		
"	"	"	6	"	"	"	"	5′30″	4′	4′45″
"	"	no. 2	2	"	"	"	"	3′30″		
"	"	"	3	"	"	"	"	3′		
"	"	"	4	"	"	"	"	3′		
"	"	"	5	"	"	"	"	3′		
"	"	"	6	"	"	"	"	2′30″	2′30″	2′50″
"	"	no. 3	2	"	"	"	"	3′		

									Optimum	*Check*
5 gtts.	plasma	no. 3	2	gtts.	$CaCl_2$	Clotting	time	3′30″		
"	"	"	4	"	"	"	"	2′30″		
"	"	"	5	"	"	"	"	3′30″		
"	"	"	6	"	"	"	"	3′	2′30″	3′15″
"	"	no. 4	2	"	"	"	"	3′		
"	"	"	3	"	"	"	"	3′		
"	"	"	4	"	"	"	"	2′30″		
"	"	"	5	"	"	"	"	2′30″		
"	"	"	6	"	"	"	"	3′	2′30″	3′30″

Antithrombin

									Average
10 gtts.	fibrinogen	+3	gtts.	thrombin	+1	gtt.	no. 1	30′	
"	"	4	"	"	1	"	"	30′	
"	"	5	"	"	1	"	"	20′	
"	"	6	"	"	1	"	"	10′	
"	"	7	"	"	1	"	"	10′	20′
"	"	3	"	"	1	"	no. 2	40′	
"	"	4	"	"	1	"	"	30′	
"	"	5	"	"	1	"	"	30′	
"	"	6	"	"	1	"	"	30′	
"	"	7	"	"	1	"	"	10′	28′
"	"	3	"	"	1	"	no. 3	30′	
"	"	4	"	"	1	"	"	30′	
"	"	5	"	"	1	"	"	20′	
"	"	6	"	"	1	"	"	20′	
"	"	7	"	"	1	"	"	10′	22′
"	"	3	"	"	1	"	no. 4	30′	
"	"	4	"	"	1	"	"	30′	
"	"	5	"	"	1	"	"	30′	
"	"	6	"	"	1	"	"	20′	
"	"	7	"	"	1	"	"	20′	26′

Experiment XII—Control. July 22, 1915. Cat 23. Weight 3.8 k. Both splanchnic nerves cut June 23, 1915.

10.10 a.m. Etherized.
10.15 a.m. Pithed.
10.39 a.m. C. T.—10′.
10.58 a.m. C. T.—9′—9′—12′.
11.00 a.m. Specimen no. 1–5 cc.—from right femoral artery.
11.31 a.m. C. T.—9′—9′30″.
11.33 a.m. Specimen no. 2–4.5 cc.
12.08 p.m. C. T.—11′—11′.
12.10 p.m. Specimen no. 3–4.5 cc.
12.24 p.m. 0.8 cc. 0.9 per cent salt solution into left femoral vein.
12.32 p.m. Specimen no. 4. 4–4.5 cc.
12.34 p.m. C. T.—5′30″—10′—10′30″.

Autopsy shows both nerves divided.

Prothrombin

									Optimum	*Check*
5 gtts.	plasma	no. 1	+2	gtts.	$CaCl_2$	Clotting	time	9′30″		
"	"	"	3	"	"	"	"	11′30″		
"	"	"	4	"	"	"	"	9′		
"	"	"	5	"	"	"	"	8′		
"	"	"	6	"	"	"	"	8′30″	8′	8′
"	"	no. 2	2	"	"	"	"	7′		
"	"	"	3	"	"	"	"	6′30″		
"	"	"	4	"	"	"	"	6′		
"	"	"	5	"	"	"	"	5′30″		
"	"	"	6	"	"	"	"	4′30″	4′30″	6′30′
"	"	no. 3	2	"	"	"	"	6′30″		
"	"	"	3	"	"	"	"	11′30″		
"	"	"	4	"	"	"	"	5′30″		
"	"	"	5	"	"	"	"	5′		
"	"	"	6	"	"	"	"	5′30″	5′	7′45″
"	"	no. 4	2	"	"	"	"	7′30″		
"	"	"	3	"	"	"	"	6′30″		
"	"	"	4	"	"	"	"	5′30″		
"	"	"	5	"	"	"	"	6′		
"	"	"	6	"	"	"	"	6′	5′30″	7′45′

Antithrombin

							Average
1 gtt.	antithrombin	no. 1	2	gtts.	thrombin	2′30″	
"	"	"	3	"	"	18′	
"	"	"	4	"	"	6′	
"	"	"	4	"	"	6′	
"	"	"	5	"	"	6′	
"	"	"	6	"	"	6′	7′30″
"	"	no. 2	2	"	"	2′30″	
"	"	"	3	"	"	6′	
"	"	"	4	"	"	6′	
"	"	"	5	"	"	2′30″	
"	"	"	6	"	"	6′	5′
"	"	no. 3	2	"	"	2′30″	
"	"	"	3	"	"	2′30″	
"	"	"	4	"	"	2′30″	
"	"	"	5	"	"	2′30″	
"	"	"	6	"	"	2′30″	2′30″

Experiment XIV. July 27, 1915. Cat 20. Weight 3 k. Both splanchnics cut June 19, 1915.

10.15 a.m. Etherized.
10.35 a.m. Pithed.
10.59 a.m. C. T—6′30″—7′45″.
11.01 a.m. Specimen no. 1—4.5 cc. from right femoral artery.

11.07 a.m. 1 cc. 0.9 per cent salt solution into left femoral vein.
11.10 a.m. C. T.—5′—5′.
11.16 a.m. Specimen no. 2—4.5 cc.
11.17 a.m. C. T.—3′30″—6′.
11.49 a.m. 0.4 cc. *adrenin* (1: 100,000) into left femoral vein.
11.51 a.m. C. T.—30″—30″.
11.54 a.m. Specimen no. 3–4.5 cc.
12.15 p.m. C. T.—2′30″—3′.
12.17 p.m. Specimen no. 4—4.5 cc.

Autopsy shows both nerves cut.

Prothrombin

								Optimum	*Check*
5 gtts.	plasma	no. 1	+2 gtts.	$CaCl_2$	Clotting time		3′		
"	"	"	3	"	"	"	4′		
"	"	"	4	"	"	"	4′		
"	"	"	5	"	"	"	3′30″		
"	"	"	6	"	"	"	3′30″	3′	3′15″
"	"	no. 2	2	"	"	"	3′		
"	"	"	3	"	"	"	2′30″		
"	"	"	4	"	"	"	2′30″		
"	"	"	5	"	"	"	2′30″		
"	"	"	6	"	"	"	2′30″	2′30	2′15″
"	"	no. 3	2	"	"	"	3′15″		
"	"	"	3	"	"	"	2′30″		
"	"	"	4	"	"	"	2′30″		
"	"	"	5	"	"	"	2′		
"	"	"	6	"	"	"	1′45″	1′45″	1′45″
"	"	no. 4	2	"	"	"	4′15″		
"	"	"	3	"	"	"	3′45″		
"	"	"	4	"	"	"	3′15″		
"	"	"	5	"	"	"	2′45″		
"	"	"	6	"	"	"	2′30″	2′30″	2′30″

Experiment XVIII. February 6, 1916. Cat 77. Weight 2.4 k. Both splanchnics cut November 10, 1915.

10.55 a.m. Pithed.
11.36 a.m. Specimen no. 1—10 cc.
11.48 a.m. 0.35 cc. adrenin (1: 100,000) into left femoral vein.
11.57 a.m. Specimen no. 2—8 cc.
12.34 p.m. Specimen no. 3—10 cc.

	SPECIMEN NO. 1 (6 cc.)	SPECIMEN NO. 2 (5 cc.)	SPECIMEN NO. 3 (7 cc.)
Weight crucible + paper + fibrinogen	13.367	13.973	13.5685
Weight cruc. + paper	*13.362*	*13.972*	*13.5680*
Weight fibrinogen	0.005	0.001	0.0005
Fibrinogen per 100 cc. plasma	0.083	0.020	0.007

The graphic representation of the results was made on a percentile basis, with the values obtained in the first specimen of blood taken as 100. For instance in Experiment VIII the prothrombin times are as follows: 4′45″, 2″50″, 3′15″, 3′30″. If 4′45″ is taken to represent the normal amount of prothrombin in the circulating blood, a decrease of prothrombin time to 2′50″ indicates according to Howell an increase in prothrombin provided the antithrombin does not vary. To express this in per cent we call 4′45″, 100 per cent. Then the relative amounts of prothrombin will be in inverse ratio to the "prothrombin times" (inasmuch as the antithrombin does not change) and the results from which the diagram of this experiment would be made would be expressed by the proportion:

$$285'' : 170'' : : x : 100$$

$$x = 168 \text{ (the nearest whole number)}$$

Thus, in the experiment, nine minutes after the injection of adrenin there was an increase of 68 per cent in the prothrombin in the circulating blood. A similar procedure was followed in calculating the antithrombin, coagulation time, and fibrinogen values on a percentile basis.

The time of each specimen was noted, taking the time of pithing as the starting point. That is, each specimen is said to have been taken so many minutes after pithing, The times of injection were noted in the same way. These varied somewhat from experiment to experiment but as the same general procedure was followed this variation was not excessive. The times that appear on the charts represent the average time after pithing when the various specimens were taken. There were usually four, occasionally five, specimens. Injections were made after the first specimen, as a rule. When two injections were made they came between the first and second and fourth and fifth specimens of blood for analysis. In averaging the coagulation times those nearest each specimen (the C. T. was done in relation to each specimen of blood for analysis) were arranged and plotted on the chart on the same ordinate as the specimen with which they were connected. The curves themselves scarcely need discussion. From figure 1 it is seen that the decrease in coagulation time is exactly proportional to the increase in prothrombin, while the antithrombin remains a straight line. Unexpectedly it was found in two experiments that the fibrinogen fell, but as was pointed out above, such minute quantities were available that these figures possess very little significance. Whether the low values obtained for fibrinogen in these animals was due to the

splanchnectomy must be determined by future work. Figure 2 shows the values obtained in the controls. As will be readily seen, there is practically no change in any of the factors.

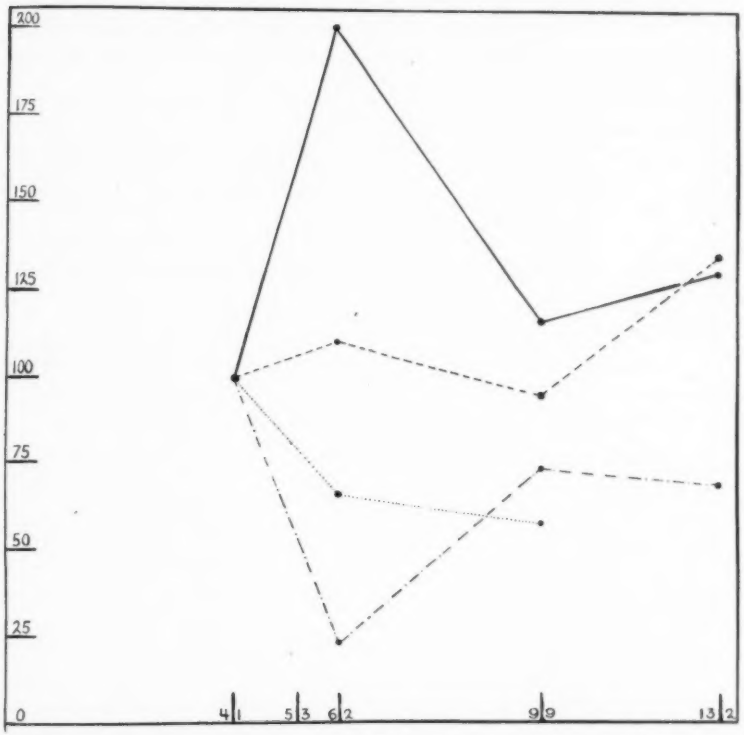

Fig. 1. Solid line represents prothrombin, dash line antithrombin, dotted line fibrinogen, dash-dot line coagulation time. Figures on the ordinate express per cent, those on the abcissa minutes after pithing. The intersection of the ordinate and abscissa indicates the time of pithing. At fifty-three minutes after pithing 0.15 cc. of adrenin (1: 100,000) per kilo was injected intravenously. These curves represent the average of Experiments vii, viii, x, xi and xiii. This figure is the graphic expression of the average percentile variation in these factors after the injection of minimal doses of adrenin. The amount in the blood of the animals before the injection was made is considered to be 100 per cent.

In comparing our results with those of Cannon and Gray it seems likely that prothrombin is elaborated in the liver and possibly in the

intestines also (2). In connection with the other actions of adrenin of biological importance, the present fact is of interest taken in conjunction with the fact that hemorrhage decreases coagulation time by decreasing the amount of antithrombin in the circulating blood (6). Under the stress of the major emotions there is a discharge of adrenin which causes a decrease in the coagulation time and under the con-

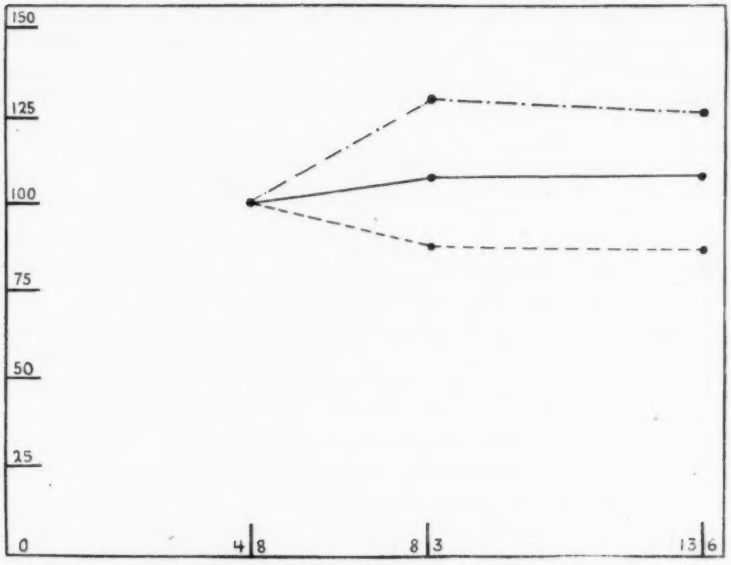

Fig. 2. Solid line represents prothrombin, dash line antithrombin, dash-dot line coagulation time. The ordinate is graduated in per cent and the abscissa measures minutes after pithing. These curves record the average variations in the factors charted of experiments iii, v, vi, xii, xiv, and xv. This figure shows the results obtained in the control experiments in some of which no injection was made and in some of which normal saline solution was injected intravenously.

ditions of savage life hemorrhage is often an accompaniment of such emotions. In such a case there would be a change in two factors of the coagulation time of the blood, each change tending to decrease this time—a powerful mechanism of vital importance to the individual.

I wish to express my sincerest thanks to Dr. W. B. Cannon at whose suggestion and under whose guidance the work was done.

CONCLUSION

The intravenous administration of minimal doses of adrenin (0.143 cc. of a 1: 100,000 solution per kilo) decreases coagulation time by increasing the amount of prothrombin in the circulating blood.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXXVI. The Physiology of the Gastric Hunger Contractions in the Amphibia and the Reptilia. Comparative Studies. I[1]

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INTRODUCTION

It is now evident from the more recent work of Cannon (1) and Carlson (2) that the sensation of hunger is due to contractions of the stomach wall in contra-distinction to the older hypotheses in existence; *e.g.*, the theory that hunger is a general bodily sensation, that it is due merely to emptiness of the stomach, that it arises from hydrochloric acid in the empty stomach, and lastly, that it is due to turgescence of the gastric glands—"turgescence theory" of Beaumont. Notwithstanding the large amount of literature upon the movements of the gastric motor mechanism, little has been done to establish the exact analogy of the gastric hunger contractions throughout the vertebrate series. Certain facts have been ascertained regarding the state of the fasting stomach in higher animals, and certain inferences have been drawn from these facts, but since the complex functions of man are unravelled many times from a study of lower forms it might be of some physiological importance if a series of similar investigations were conducted on the remaining classes of the vertebrates and even perhaps upon certain forms of the invertebrates. Up to the present time, however, all the principal work has been done on a single animal group,

[1] A preliminary report of this work was made before the 1915 meeting of the American Physiological Society at Boston, a brief abstract of which was published in the Proceedings of that society. Also some of the results were presented in a paper read before the Kingston Medical and Surgical Society, February 28, 1916.

the mammalia, with the exception of some work now in progress on the group of the aves. Therefore, in descending the vertebrate scale to new fields of investigation in this particular phase of physiology the next groups in order are the reptilia and the amphibia, respectively. Representatives from these two groups will be dealt with in this report.

Various investigators have worked with the spontaneous movements of the gastro-neuro-muscular apparatus of poikilothermic animals which has been confined principally to the frog under varying conditions. They have studied the excitatory and the inhibitory influences upon gastric motility through its extrinsic innervation by means of electrical, mechanical and chemical stimuli, as well as the direct effects on this organ. With regard to the gastro-inhibitory effect of the vagus certain experiments upon the frog are of special interest, Volkmann (3) in 1841 found that if the medulla oblongata is destroyed or the vagi are cut, a lively peristaltic movement in the stomach is observed. Goltz (4) in 1872 confirmed the above results, but neither of these authors was able to correctly interpret his findings; the former could not yet think of inhibition, and the latter although the interpretation suggested itself could not conceive of the idea that inhibitory and motor-nerve fibers could be contained in the same nerve trunk. The appearance of a movement after the section of the vagi, in a stomach previously at rest, seems to indicate that in a normal state these nerves exert an inhibitory influence upon the stomach. The results obtained by Goltz (5) from curarized frogs which had been left unfed for some days previous to the beginning of the experiment showed in addition, that the automatic movements of the frog's stomach disappeared after the destruction of the brain and cord, as well as after the cutting of both vagi. According to this observation the vagi would contain inhibitory fibers for the stomach, but, since the gastric wall remained in a more or less tonic condition and was not followed by expansion as far as I am able to determine from a review of Goltz's paper, the movements of the frog's stomach itself must have been suppressed by the artificial excitement of the vagi. Furthermore, he states that the mere cutting of the vagus with scissors in close proximity to the esophagus gives rise to violent movements in the wall of the stomach. This would indicate the existence of motor fibers for the frog's stomach. The presence of gastro-inhibitory fibers in the vagi have been conclusively shown by Langley (6), Meltzer (7), May (8), and Cannon (9) in mammals. According to Kronecker and Meltzer (10) the tonus of the cardia for the rabbit at least is diminished or

inhibited at the onset of each deglutition, and if many deglutitions follow one another in rapid succession, the tonus may be entirely abolished. This relaxation of the cardia is of central nervous origin since it takes place even when the esophagus is severed, but disappears when the vagi are cut. Auer (11) has reported that only a slight degree of reflex inhibition of the stomach is obtained through the vagi in rabbits. Von Openchowski (12) has described nerve twigs of the vagi in the proximity of the cardia, from one of which he obtained by stimulation contraction, and from another relaxation of the cardia. According to May (8, 13) the vagi contain both motor and inhibitory fibers for the stomach musculature. Stimulation of the vagus produces two effects, namely, an inhibitory or dilatatory effect followed by an increase of gastric muscular tone and movements, the former predominating in the cardiac end of the stomach and the latter in the pyloric end. Similar effects were produced by "anemia" of the stomach when it was suddenly produced. This author also believed that the splanchnics had no direct influence either motor or inhibitory on the stomach muscular wall. Later investigations by Cannon and Murphy (14) and Auer (11) have shown that the inhibition of the gastric movements of digestion is produced through the splanchnic nerves and the same has been verified by Carlson (15) for the gastric hunger contractions. Furthermore, it has been shown by Cannon (16) for the digestive movements and by Carlson (15) for the hunger movements that the gastric tonus is largely maintained through the vagi. Mayer (17) has described three motor effects resulting from vagus excitation in the neck of rabbits, namely, (*a*) The cardial part of the stomach becomes very stiff, then follows a subsequent contraction-pyloric wave. (*b*) The usually observed depression between the cardial and pyloric portions becomes more manifest and depressions in other parts are noted. (*c*) The whole stomach musculature passes into a state of tetanic contraction which persists a long time. Longet (18) did not obtain such results from vagus excitation in the neck of this animal. The discrepancy in the results was due to the fact that in the period of digestion the above mentioned effect of the nerve action is not manifested. Adrian (19) verified by electric excitation of the plexus coeliacus and its neighborhood, movements in the cardia and pylorus.

The experimental work in connection with the excitatory and inhibitory movements of the frog's stomach has not been confined alone to the vagus. Various other nerves under stimulation have been found to exert a marked influence on the gastric motor mechanism. Waters

(20) noticed that strong stimulation of the third, fourth, fifth and sixth nerves in the frog at their exit from the spinal cord led to contractions of the stomach, as well as the esophagus and sometimes the small intestine. Steinach and Wiener (21) have described motor effects in the frog's viscera as a result of stimulation of the posterior roots. According to these authors the stomach is innervated from the third, fourth and fifth spinal nerves. Dixon (22) verified the above results by stimulation of the rami communicantes of the third, fourth and fifth spinal nerves which corresponds to the origin from the spinal nerves described by Waters and Steinach and Wiener. According to this author stimulation of the rami communicantes of the fourth spinal nerve produces tonic contraction together with augmented and more regular automatic stomach contractions. Stimulation of the third and fifth rami produces a similar but smaller effect. The vagus contains inhibitory fibers to tonus but upon excitation of this nerve augmented automatic movements usually result, but no increase in gastric tonus is ever observed. Contejean (23) in studying the innervation of the stomach of Batracians found that electrical excitation of the sympathetic caused no relaxation of the gastric reservoir, it even caused contraction although to a lesser degree than excitation of the vagus. It is with the vagus and not with the sympathetic that this author has been able to provoke arrest phenomena in the peristaltic movements of the stomach, and he thinks the vagus acts especially on the longitudinal fibers while the sympathetic commands the circular fibers although they are probably not absolutely dependent upon the sympathetic. He has also quite regularly provoked contraction of the pylorus in dogs by electrization of the vagus. Meltzer (24) found that very weak excitation when applied directly to the stomachs of dogs, cats, rabbits and frogs caused contraction, if the electrode was placed on the outer side of the stomach. This varied somewhat with the animal used, but the main excitatory parts according to him were the pylorus, the cardia and slightly the fundus, except in the case of the frog in which these points were not definitely localized. Battelli (25) studied the gastric movements under the influence of drugs, and draws the interesting conclusion that the vagus by the inner branch of the accessorius not only regulates the moving phases but also the inhibition phases of the stomach muscles. Doyon (26) found that excitation of the peripheral end of the vagus under ordinary conditions in dogs undergoing digestion, the nerve of the opposite side being intact, invariably provoked exaggeration of the stomach movements, but he

never observed any clearly marked suspensory action of the nerve on the gastric reservoir or a diminution of the gastric tonus. According to this author an injection of pilocarpine or strychnine appears to favor the putting into play of the inhibitory power of the vagus nerve on the movements of the stomach, since excitation of the peripheral end of this nerve after the injection causes a decontraction of the gastric reservoir followed by an exceptionally energetic contraction and he suggests that these substances provoke a combined effect through the gastro-inhibitory and gastro-motor fibers of the vagus. Barbéra (27) used the stomach sack method in investigating the spontaneous activity of the frog's stomach. The brain was separated from the medulla and two cannulas were introduced into the stomach; one into the cardia and held by an esophageal ligature, and the other through the pylorus and fixed by a duodenal ligature. The stomach was then filled with normal saline solution at a small pressure of from 3 to 4 cm. in the ascending cardial orifice and connected with a Marey apparatus for registering the movements or alternations in its internal capacity. With such an arrangement Barbéra studied the effect of electrical excitation on the gastro-spontaneous movements, and concluded that the stomach muscular movements were reflex movements. Gläfsner (28) with a somewhat similar arrangement studied the effect of different substances (poisons) in the stomach. He used a double cannula instead of two single cannulas which he introduced through the esophagus. Dixon (22) undertook a similar inquiry but used an internal pressure of from 10 to 20 cm. up in a frog's stomach. He thought that the optimal water pressure varied greatly with different animals and emphasized the fact, that the spontaneous movement with his method exhibited greater regularity.

There were generally differences of opinion as regards the animals among authors. Some thought that regularity and strength of the spontaneous contractions varied according to the animal, and that in the case of frogs, winter frogs were not suitable. Also adverse ideas existed as regards unfed and fattened frogs. Morishima and Fujitani (29) investigated the spontaneous movements of the frog's stomach (Esculenta) with a William's heart apparatus. They isolated the whole stomach in toto, but used only the part in the neighborhood of the pylorus, so that, when the double-walled cannula was tied in the fundus the prepared "Magensack" constituted about one-third of the whole stomach wall. The pyloric end was closed by a ligature and the whole placed into Ringer's solution into which a slow current of

oxygen was led. These authors verified that regular and strong contractions are obtained with well fed frogs, and they do not think that the condition of the stomach, that is, full or empty is of great consequence. Gl ifsner (28) used either freshly caught hungry frogs, or those confined a long time, fed on rain-worms, etc., and then left without food for some days before the experiment. Dixon (22) thought that hungry frogs such as Gläfsner and others used gave much worse results than normal animals, and that if kept in confinement for two months were quite unfit for use. According to Morishima's and Fujitani's experience the stomach filled with food is the best for studying spontaneous movements, but in case of frogs kept a long time in confinement better results are obtained if frog-muscle or rain-worms are fed them some days before the experiment. More recently, Hopf (30) with a similar arrangement to Barbéra's has shown conclusively that stimulation of the vagus in the frog can have the effects of inhibition and of excitation on the gastric reservoir. In order to obtain g od results the stomach must be placed in a good condition of automatic contraction (motility). The phase of inhibition is then seen preceding that of excitation, and the excitation is stronger than the inhibition, the intensity and the duration of the current having little or no influence on the vagus effect. According to this author there is a great distinction between fed and unfed frogs in the size and stability of the automatic stomach movements, but winter frogs give good results if previously fed.

EXPERIMENTAL PROCEDURE

The comparative studies on the amphibia and the reptilia were made on the bullfrog (Rana catesbiana), better known in the fish markets as the "jumbo frog," and the common snapping turtle (Chelydra serpentina), respectively. All the bullfrogs were obtained from the South, Louisiana, through local dealers in Baltimore and Chicago and were stomostomized. This simple operation which has only been briefly described by the author (31) consists of making a circular opening on one side between the ramus of the inferior maxillary near the posterior angulosplenial region and the anterior cornua of the hyoid bone through the skin, the submaxillaris (mylohyoideus) muscle and the lining membrane of the pharyngo-oral cavity (32) of sufficient size (about 8 mm. in diameter) to admit the balloon and the attached rubber tube which connects with the recording manometer. This operation may be bloodlessly performed provided care is taken to avoid injury

to the superficial mandibular vein (vena maxillaris inferior) which runs along the insertion of the submaxillaris muscle, and turns inwards at its hinder border to join the lingual vein. Through this artificial opening or stomostomy which usually heals in from three to four days so that the animal may be used for experimental tests with normal results is introduced the delicate rubber balloon by means of a glass seeker. Then by opening the frog's mouth the balloon may be carefully pushed into the stomach with the seeker through the short esophagus which may be greatly enlarged, yet when empty is completely closed by folding of the walls. In this position the balloon may be inflated through a glass T-tube and the desired pressure obtained in the manometer, the rubber tube passing through the stomostomy to which the balloon is attached lying in the posterior part of the pharyngo-oral cavity under the free edge or at the side of the tongue, and thereby eliminating any possibility of the stomach pressure fluctuations on the balloon which are transmitted by air transmission to the surface of the liquid in the manometer being partially or entirely shut off, which would be the case, if the flexible rubber tube passed between the jaws so the animal could bite upon the tube. Like the gastric fistula in dogs there is no trouble in the way of closing up of the stomostomy as long as the animal is being used daily, but when unused it usually closes up completely in from five to seven weeks. The opening appears to be of practically no inconvenience to the animal. It will have to be admitted that practically all the methods previously used by investigators for studying the spontaneous movements of the frog's stomach were more or less pathological in their entirety, while the method used in this investigation allows the animal to remain in a perfectly normal condition at all times, since the stomostomy in the floor of the mouth does not interfere in any way with the normal physiological activity of the gastric motor apparatus or any other system of the body. The large species of frog is also advantageous with this method since more accurate results are usually obtained from larger animals.

During the record taking of the gastric hunger movements, the animals were placed individually in small laboratory table sinks, 7 by 10 inches and 5 inches in depth. The bottoms were covered with several layers of filter paper to partially close the outlet and water was allowed to drip very slowly from the faucet. Each sink was then covered with the exception of a very small opening for the passage of the rubber tube from the balloon in the frog's stomach to the manometer. With this arrangement the animals were practically con-

cealed from all disturbing influences and the sinks, darkened as they were by opaque covers, the animals felt themselves securely hidden and would remain very quiet for long periods of time. Two animals were always run at the same time, the one as a control on the other, and the same tests were always applied to both. For the temperature experiments it was necessary to use different devices. To determine the effects of raising the temperature upon the movements of the gastric hunger apparatus, the animal was placed in a glass cylinder of $7\frac{1}{2}$ inches diameter and 6 inches in height, having a $2\frac{3}{4}$ inch raised base of wire gauze covered with a circular galvanized iron plate, the size of the interior of the cylinder. The animal stood upon the raised base and occupied the upper $3\frac{1}{4}$ inches of the cylinder. The top of the cylinder was provided with a wooden cover containing two small openings, one for a thermometer, and the other for the rubber tube from the balloon. Around the glass cylinder was wrapped a dark colored wet towel to darken its interior and the whole was placed in a large vessel containing water at ordinary room temperature to a height that rose within about $\frac{1}{2}$ inch of the galvanized iron plate within the cylinder. The temperature of the water was now very gradually increased at the rate of about 10°C. per three hours and continuous records of the hunger movements recorded. On the other hand, the effects of cooling or lowering the temperature was determined by placing the animal in a box 12 by 10 inches and $8\frac{1}{2}$ inches in height, the sides of which were covered with $\frac{1}{4}$ inch mesh wire gauze and the top and bottom of wood. Around the sides and top was placed a dark colored wet towel to darken the interior as in the case of the cylinder above, as well as to shut out all possible disturbing influences. The animal so arranged was placed in a refrigerator, the door of which having been previously left open until the temperature of its compartment reached approximately that of the ordinary room temperature. The temperature was obtained by a thermometer in the box which was very gradually lowered by the gradual closing of the refrigerator door at the rate of about 10°C. per three hours, while continuous records were being taken. However, in one case where it was desired to reduce the temperature to a very low degree, a freezing mixture of cracked ice and salt was packed around the glass cylinders above described and then the animals placed in position and connected with the recording apparatus. As might be expected, this did not give nearly as satisfactory results as did the refrigerator method, since the temperature was reduced too rapidly. However, this very low temperature did

bring out certain additional facts of rather great importance which the other method did not.

In the species of turtles used the plastron is small and narrow, exposing a great amount of the fleshy parts, therefore it was possible to perform a gastrostomy on these animals. An incision about 3 inches in length was made near the posterior curvature of the plastron of the left side through the abdominal wall, and the ordinary procedure then followed as outlined by Carlson for gastrostomy on dogs (33). As a rule, snapping turtles are very vicious and are rather unique among chelonians in defending themselves in a similar manner to snakes, that is, by "striking" at the object of anger. The rapidity with which the head is shot forward when these animals are disturbed rivals the dexterity of the rattlesnake, and when we consider that they are provided with a pair of keen-edged, cutting mandibles and jaw muscles of tremendous power, the stroke of these dangerous brutes may be followed by anything but superficial injury. In fact the amputation of a finger by a medium-sized specimen, or a hand by a very large individual would be an accomplishment of no difficulty to the reptile (34). Therefore great precaution must be used in handling these animals for experimental purposes or otherwise. The safest way to handle a large specimen is to pick it up by the tail and hold it well off from the body, but I have found it also quite safe to handle it by the posterior lateral portions of the shell. The animals used weighed from 10 to 15 pounds, and the hunger contractions were recorded by a balloon introduced through the fistula into the stomach. During the recording of the contractions the animals were placed in a specially constructed box, $17\frac{1}{2}$ by $10\frac{1}{2}$ inches and $4\frac{1}{2}$ inches in depth and supported on legs 13 inches in height, in the bottom of which was an oval opening 7 inches in length by $4\frac{1}{2}$ inches in width. This opening in the bottom of the box gave access to the fistula for the introduction of the balloon into the stomach and removed all possible danger of injury from the vicious animals. This box was provided with a lid at one end covered with wire gauze and was of such size that the animal was comfortable and could move about slightly, although the internal capacity was not sufficiently large to permit the animal to turn completely around. With this arrangement the animal after it had once quieted down would remain quiet for hours and continuous records of the gastric hunger movements could be obtained with ease. All the records from the frog and turtle were taken on a slowly moving drum revolving at the rate of about fifty minutes per revolution.

THE CHARACTER OF THE GASTRIC HUNGER MOVEMENTS FROM THE EMPTY STOMACH OF THE FROG

The recorded observations upon the activity of the gastric hunger mechanism of the frog have numerous points of dissimilarity as compared to the numerous observations already on record for higher animals. Thus, Carlson (35) has demonstrated that the empty stomach during prolonged fasting exhibits three types of motor activity, namely:

1. Rhythmical contractions of about twenty seconds' duration and designated the "twenty second rhythm."
2. Very strong contractions occurring periodically of about thirty seconds' duration and designated the "thirty second rhythm."
3. Tonus changes of the stomach musculature.

In the frogs under observation there were no tonus changes observed, not even in prolonged fasting. This would seem to confirm the work of Dixon (22) on the vagus of the frog, namely, that excitation of this nerve causes no increase in gastric tonus although augmented automatic movements usually occur. Since the different types of these hunger contractions are dependent upon the degree of tonus of the stomach and since there are no tonus changes in the frog's stomach it would be expected that only one type of hunger contraction would be exhibited by this animal, and this is found to be the case. This particular type of contraction shows an average duration of about one and three-fifths minutes, and the intervals between the contractions vary from sixteen to thirty-three seconds. These contractions are very powerful and are evidently analogous to the thirty seconds rhythm in man and dog, type I contractions, but the individual contractions are very much more vigorous, that is, if we take into consideration the body weight of the frog as compared to the body weight of the above mentioned animals. In fact, they even excel in strength and amplitude, the most powerful contractions from either man or dog in prolonged fasting (fig. 1). In general appearance the contraction is rather slow, the curve is perfectly smooth and shows no smaller superimposed waves, and there is no indication that the contractions fall into groups, separated by intervals of relative quiescence. In the higher animals the hunger contractions are periodic and represent simply more vigorous peristaltic movements during which the stomach becomes markedly hypertonic (40, 52). On the other hand, the gastric hunger con-

tractions of the frog are continuous and show a definite regularity which goes on indefinitely hour after hour and day after day with no periods of rest except for the few seconds' pause between each individual contraction. This corroborates the work of Morishima and Fujitani (29) on the frog (Esculenta) that very regular curve tracings may be taken hourly, eventually ten hours, and they have

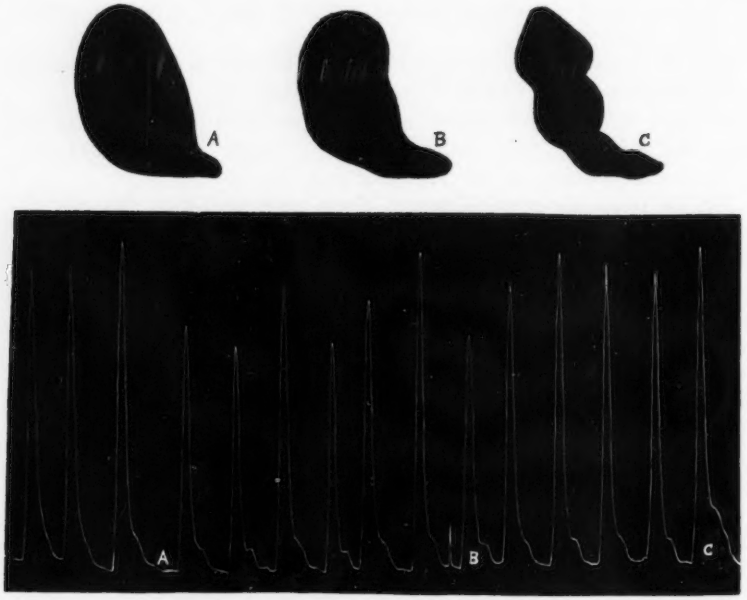

Fig. 1. Hunger contractions of the frog's stomach after twelve days' fast. *A*, outline of stomach (as seen by X-ray and bismuth balloon), between hunger contractions with stomach relaxed. *B*, outline of stomach at the height of a moderately strong hunger contraction. *C*, outline of stomach at the height of a very strong hunger contraction. Water manometer.

published a series of these normal curves selected at definite intervals which extend over a period of about eight and a half hours from the beginning of the experiment. This corroboration is all the more valuable, as the method employed by these authors is totally different from the one used by myself. It was also found that when the pressure in the balloon in the frog's stomach was increased that it

increased the pressure in the manometer only momentarily, the stomach simply dilating to accommodate itself to the increased size of the balloon. This may be repeated several times with apparently no change in the contractions or increase in the manometric pressure except temporarily, but finally a limit of stomach expansion will be reached in which to further increase the pressure by blowing reasonably will be impossible and at the same time the manometric pressure will be markedly increased. This no doubt, is an adaptive accommodation which this low form of vertebrate animal possesses, since the periodic habit of feeding is unformed and when food is abundant it eats greedily, the stomach dilating to act as a reservoir for the food.

THE CHARACTER OF THE GASTRIC HUNGER MOVEMENTS FROM THE EMPTY STOMACH OF THE TURTLE

The gastric hunger movements of the turtle's stomach show an advance in physiological development over the gastric hunger activity of the frog, and conform more to the results obtained from the stomachs of higher animals. In fact, this would be the natural thing for us to expect considering the different positions that these two animals occupy in the animal scale. Slight tonus variations are observed and two distinct types of hunger contractions may be described in the turtle. The first type of contraction in this animal (fig. 2, *A*) shows an average duration of about one minute, and the intervals between the contractions vary from three to three and a half minutes. These contractions at first appear to be less vigorous than those from the frog's stomach and perhaps for this reason they are slightly shorter in duration. This type of contraction is characteristic of the early part of the hunger period and is no doubt analogous to the thirty seconds rhythm in man and dog, type I contractions. It also corresponds to the type I contractions described for the frog. The small irregular waves which appear here and there in the rest intervals between the individual contractions are exaggerated respiratory movements. The gastric contraction of the turtle like that of the frog is rather moderate in appearance and the curve is perfectly regular with no indication of smaller waves superimposed upon it. In the latter part of the active hunger period the type I contractions above described gradually change into a second type of contraction which tends to approach incomplete tetanus at every

Fig. 2. Hunger contractions of the turtle's stomach after twenty-one days' fast. *A*, initial forty minutes of a five and one-half hours' typical period of hunger contractions. *B*, final thirty minutes of the same active period. At *X* the active period terminates and the stomach passes into a period of quiescence. Note in tracing *B* the incomplete tetanus. Water manometer.

regular periodic contraction of the stomach and this effect becomes more marked as the contractions continue. There is also an increase in gastric tonus during each contraction with its tetany and usually a slight but general increase in the whole tone of the stomach musculature (fig. 2, *B*). This second type of contraction is probably the primitive ancestor of the twenty seconds rhythm in man. The contraction with its incomplete tetanus shows an average duration of about two minutes, and the intervals between the contractions in this case vary from three and a third to four minutes. The hunger contractions of the turtle unlike those of the frog but resembling those of the higher animals fall into groups of gastric activity, separated by intervals of relative quiescence. The duration of the periods of gastric hunger activity vary from five and a half to six hours and the intervening periods of quiescence of the stomach from one to one and three-quarters hours, with but one exception in which the quiescent period was found to be of four and one-sixth hours' duration. The period of gastric hunger activity in the adult turtle appears to be relatively long as compared to that of an adult dog (36) which ranges from one and a half to three hours in length but again when we stop to consider the place of this animal in the vertebrate scale, we would expect it to take an intermediate position and that the gastric activity would also be intermediate between the frog and the higher animals, and this is evidently the case. The gastric motor mechanism of the turtle shows a physiological development above the state of a simple, continuous contracting organ so characteristic of the stomach of the frog, yet it has not become sufficiently developed to be classed physiologically with the stomachs of higher animals, and therefore must play the important part of a connecting link in the gastric motor mechanisms of animals. The stomach of the turtle like that of the frog also exhibits the property of expansion and dilatation as determined by increasing the pressure in the balloon.

THE INHIBITION OF THE GASTRIC HUNGER CONTRACTIONS IN THE FROG AND THE TURTLE

It is now demonstrated that the tonus and the hunger contractions of the empty stomach in man and dog are temporarily inhibited by mechanical and chemical stimulation of the nerve endings in the mucous membrane of the mouth, in the esophagus, and in the gastric mucosa (37). Similar stimulation when applied to the intestinal mucosa

also produces the effect of gastric inhibition (38). This inhibition is initiated by stimulation of nerve endings in the gastric mucosa, and not by mechanical tension or pressure on the stomach wall. The results on normal frogs compare generally with those on man and dog already reported with some few exceptions. When water, sodium carbonate—1 per cent solution and hydrochloric acid—0.5 per cent solution are introduced very slowly into the empty stomach of the frog, through a small rubber tube passing through the stomostomy, they invariably produce inhibition varying in degree with the stimulating power of the substance introduced. The duration of the inhibition depends upon the quantity and nature of the material introduced into the stomach and not so much upon the degree of the hunger contractions as in the case of the higher animals, since there are no tonus changes and only one type of contraction exhibited by this animal. In normal frogs 5 to 10 cc. water introduced directly into the stomach via tube usually produces a weakening of the contractions (fig. 3, *A*) but never in any of my experiments have I witnessed complete inhibition of the gastric movements. The partial inhibition is seen to come on very gradually as indicated by a lessening in the amplitude of the hunger contractions which usually extend over a period of from ten to fifteen minutes followed by a gradual recovery to normal. The water so introduced was at ordinary room temperature and should correspond approximately to the temperature of the frog's body, therefore the water in the stomach must have produced the temporary inhibition through a stimulation of the nerve endings in the gastric mucosa either by mechanical pressure or by osmosis. According to this view, the passage of the water out of the stomach into the intestine, or the addition of sufficient salts to prevent stimulation by hypotonicity would mark the cessation of the inhibition. It was also found that the inhibitory action of cold water was slightly greater than that of water at the temperature of the frog's body, but in order to bring out this phenomenon effectively it was necessary to use very cold water, which evidently stimulated the protopathic temperature nerve endings in addition to those acted upon by pressure and osmosis. The results overbalance any error that might be accounted for by the cold water cooling the air in the balloon and thereby temporarily lowering the tension in a very small degree. The introduction of 5 cc. sodium carbonate, 1 per cent solution directly into the stomach produces a more marked inhibitory effect than does the water, the contractions may be greatly weakened or they may be completely inhibited for one and a half

to two minutes (fig. 3, *B*). Five cubic centimeters hydrochloric acid, 0.5 per cent solution causes complete inhibition of the hunger contractions for periods ranging from seventeen to twenty-five minutes (fig. 3, *C*). The duration of the inhibition in the case of acids and alkalies seems to be directly proportional to their concentration and the total

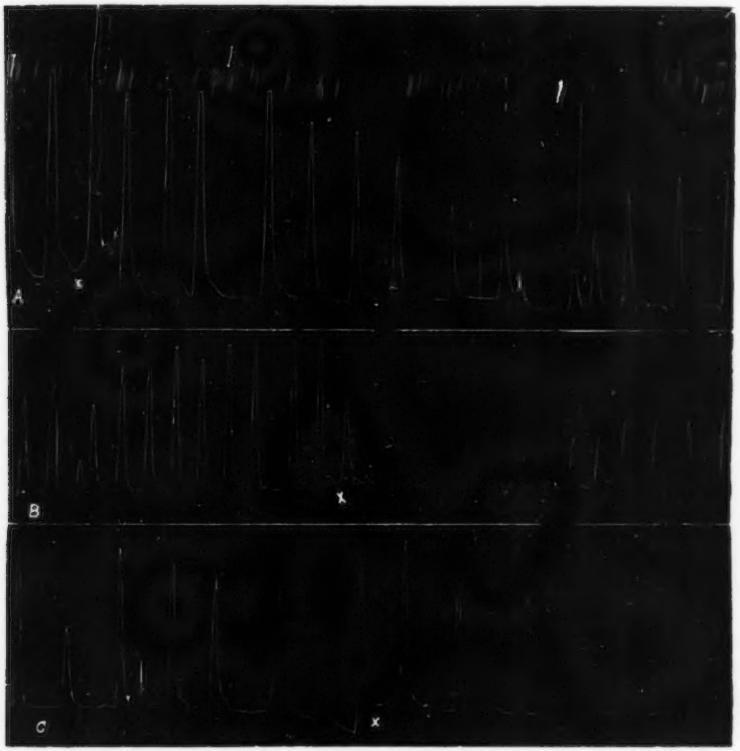

Fig. 3. Records from the frog's stomach after fourteen days' fast showing the inhibitory effects produced by different substances when introduced into the stomach. *Tracing A:* At *X* 10 cc. water introduced directly into the stomach. (The slight drop in tonus after *X* was due to bubbles of air escaping through the manometer.) *Tracing B:* At *X* 5 cc. of 1 per cent solution sodium carbonate introduced directly into the stomach. *Tracing C:* At *X* 5 cc. of 0.5 per cent solution hydrochloric acid introduced directly into the stomach. Water manometer.

quantity introduced, it being the most marked always in the case of the acid. The cessation of the inhibition by these substances probably marks the passage of the same into the intestine or their fixation and neutralization. The inhibition of the gastric contractions in the frog varies from that of the dog and man in that they are not immediately produced after the introduction of the substance into the stomach, but there is usually from one to four or five strong contractions before the inhibition becomes complete. This would seem to indicate that the gastro-reflex apparatus of the frog had not reached the high degree of efficiency found in the stomachs of higher animals. The above mentioned substances when introduced directly into the stomach of the turtle via small rubber tube through the fistula produced the same general inhibitory effects as in the frog.

When water, alkali or acid of the above concentration and in amounts as used for the stomach were introduced directly into the mouth cavity of the frog through a small rubber tube which just passed through the stomostomy into the mouth it was found that the inhibitory effects produced were very slight and perhaps might even have been negative altogether were it not for the fact that some of the solution probably finds its way immediately to the stomach and there may produce inhibition by coming in contact with the gastric mucosa. These observations on the difficulty of inhibiting the hunger movements from the mouth are in agreement with those reported by Rogers (39) for the rabbit and by King (40) for the guinea-pig, except in these two animals the reflex inhibitory mechanism of the stomach from the mucous membrane of the mouth appears to be entirely absent, since these authors have obtained only negative results. The above mentioned substances when placed into the mouths of higher animals (dog and man) produces a marked inhibition of the gastric hunger movements reflexly, therefore it would be reasonable to believe that the cerebral processes are not as highly developed in these animals, the frog included as in the case of man and the dog. It was impossible to try any of these experiments on the turtles because of the viciousness of these animals. Reflex or psychic inhibition of the hunger contractions in both the frog and the turtle was obtained by showing these animals a small live grass frog on a covered glass plate. This agrees with the results obtained from dogs that the sight or smell of food leads to temporary inhibition of the gastric hunger contractions. Anything which frightens, annoys or angers these animals also leads to temporary inhibition of the contractions.

THE MOVEMENTS OF THE FILLED STOMACH AS COMPARED TO THE HUNGER MOVEMENTS AND THEIR INHIBITION IN THE FROG

In the higher animals (dog and man) there is a marked distinction between the gastric movements in normal digestion and the gastric hunger movements of the empty stomach. Acids in concentrations equal to that of the gastric juice when introduced directly into the stomach do not inhibit the movements of the stomach in digestion but will inhibit the hunger contractions (41). In the rabbit (39) and the guinea-pig (40) these two types of gastric contractions are less sharply differentiated, although during fasting the stomach activities may be greatly augmented. In the case of the rabbit the inhibitory action of acid gives the same general results as for the higher animals, with the exception that the effect appears to be less pronounced. In the guinea-pig, according to King, acid placed in the stomach gives negative results.

Since the cannibalistic traits of the bullfrog are well known (42) it is a very easy matter to obtain these animals with a filled stomach, and furthermore, if the animals are kept in separate compartments, the amount of food can be controlled at any one feeding. The animal when hungry usually captures its prey (small frog) by the anterior portion of the body as soon as it strikes the water and swallows it alive, and I have observed one of these animals several times to devour as many as four adult grass frogs in as many minutes and have watched with intense interest the struggling of the smaller frogs in the stomach of the larger. At times they would appear to crawl up into the esophagus when it would become necessary for the larger frog to swallow vigorously several times in order to keep them down. However, these animals are soon asphyxiated since the large esophagus is completely closed by foldings of its substance and therefore effectually prevents any accession of air into the stomach. The frogs for the experiments were usually fed two live grass frogs each, and after a period of from thirty minutes to one hour in order to allow sufficient time for asphyxiation the balloon was introduced into the stomach and connected with the recording apparatus. Records of the frog shortly after feeding (fig. 4, *A*) when compared with the normal hunger records (fig. 1) show but very little change, if any, from the stomach of the hungry animal, the only observable variation being perhaps a very slight increase in the rate of the contractions. This is in confirmation with the view put forth by Morishima and Fujitani (29) that the stomach, full or empty,

is not of great consequence as regards the regularity and intensity of the spontaneous movements, although they did not offer sufficient proof to uphold their hypothesis. These facts are contradictory to the ideas of Hopf (30), who claims that there is a great distinction between fed and unfed frogs in the size and stability of the automatic stomach

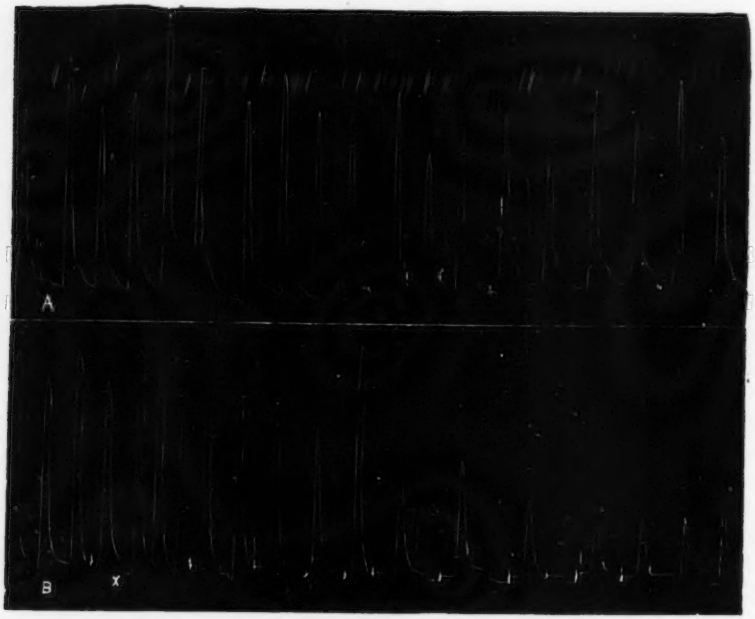

Fig. 4. Normal digestive peristalsis of frog's stomach. *A*, 3 hours after feeding two small live grass frogs. *B*, same animal 10 hours after feeding, food still in stomach. At *X* 10 cc. water introduced directly into stomach producing temporary inhibition. Water manometer.

movements, and it is probable that the method which he employed was partly at fault and pathological.

The question arose in respect to how long it required for the stomach to empty itself after feeding. This was determined after feeding the "jumbo" one small frog and then examining from time to time the stomach contents by inserting a pair of forceps through the mouth and esophagus into the stomach and exposing the digesting animal. I was also interested to find in reviewing the literature after completing

the experiments that this same method was used by John R. Young (43) of Maryland in 1803 in determining the rate of gastric digestion in the frog as a part of a medical dissertation submitted to the University of Pennsylvania. However, after some experience I could determine by palpating over the region of the stomach as to its condition and I found by both of these methods that it required from forty-eight to sixty-eight hours for the stomach to completely empty itself. With larger feedings of two, three and four small frogs, the stomach would evidently require a longer period to empty itself. Young did not determine the total time necessary for the stomach to completely empty itself, he simply studied the rate of digestion. In regard to the introduction of water, alkali or acid it was found that they produced similar inhibitory effects in both the hungry and the filled stomach of the frog (fig. 4, *B*) whereas in the case of the higher animals, these same substances inhibit the hunger contractions but have no effect upon the gastric peristalsis with but one exception, namely, in the guinea-pig in which these substances have no effect whatever on either the hunger or the peristaltic movements. This is a little out of harmony with the results obtained from other animals and one would naturally expect from a comparative standpoint that this animal from the position it occupies in the animal scale should possess a reflex inhibitory mechanism from the gastric mucosa. King (40) states that she had difficulty in introducing substances directly into the stomach but in a few instances succeeded in introducing about 1 cc. of the various substances. Had she introduced more of these substances would she not have brought out the reflex inhibitory mechanism of this animal? From the above results it would appear that in the frog, at least, we have a much simplified gastric mechanism which through the processes of evolution has in the higher animals differentiated into the gastric digestive peristalses and the gastric hunger contractions, the latter of which perhaps may be described as intensified gastric digestive peristalses. Thus, the automatism of the gastric mechanism of the various classes of vertebrates apparently stands in correlation with the degree of development and influence of the central nervous system.

THE EFFECT OF PROLONGED FASTING ON THE GASTRIC HUNGER MOVEMENTS

In prolonged fasting the stomach activities in all mammals so far studied are greatly augmented and show without exception marked tonus variations in the stomach musculature. In dogs during pro-

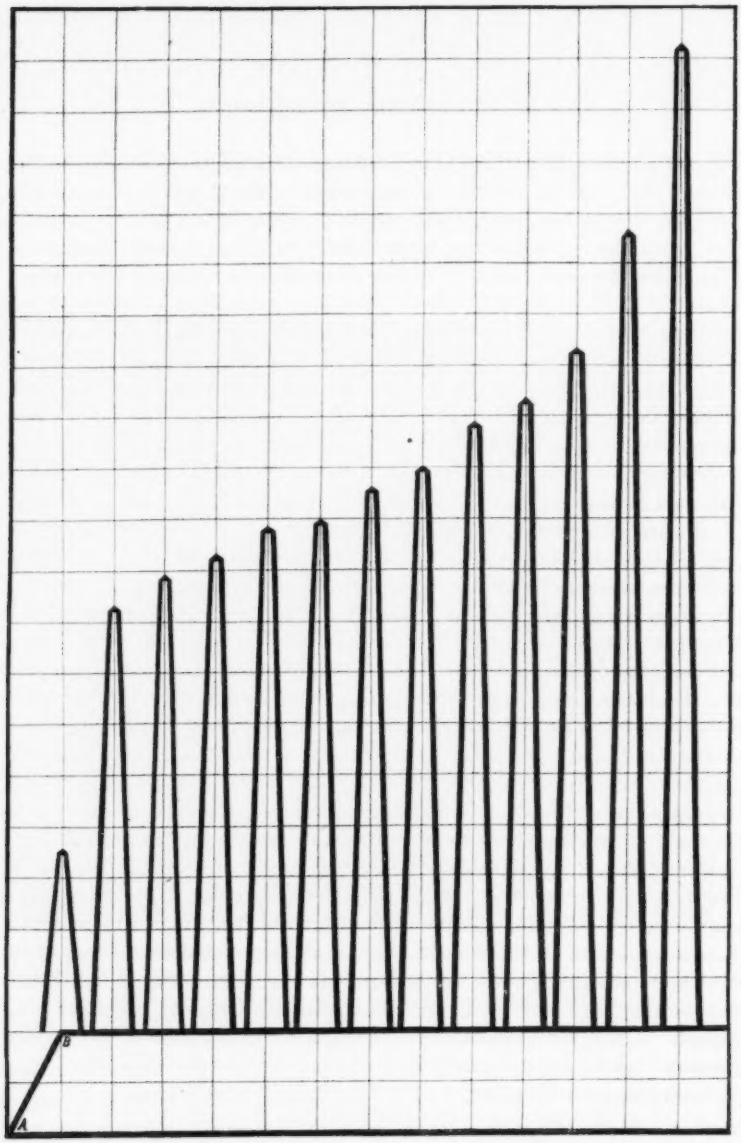

Fig. 5. Diagrammatic representation of the increased amplitude of contraction on the base of the constant tonus as constructed from the tracings of the turtle on every fifth day. Each of the above squares represents one sq. cm. The erect pyramids indicate the amplitude of the hunger contractions in centimeters arranged on the constant tonus as a base line. Spaces left to right indicate periods of five days' fasting. Heavy line at bottom of chart = 0 mm. pressure of water manometer. *A* to *B* = constant pressure of 2 cm. used throughout the experimentation. Note the enormous increase in the amplitude of the hunger contractions at the end of the 64 days' fast.

longed fasting there is a marked increase in the gastric tonus to within a few hours of the death of the animal, or at least, until that point is reached where the stomach becomes involved in the general debility and cachexia, and this increase in the gastric tonus appears to be directly proportional to the decrease in the amplitudes of the hunger contractions (44). In the turtle there are only slight tonus variations while in the frog there are no tonus variations of sufficient strength to be recorded by my apparatus. There is no general increase in the gastric tonus in the turtle in prolonged fasting but we have instead a marked increase in the amplitudes of the hunger contractions with no appreciable increase in the gastric tonus as is shown by the accompanying chart (fig. 5). The same results are indicated in the frog although this animal was not subjected to such long periods of fasting. These results are just the reverse of what is found in the case of the dog. The longest fast on the turtle covered a period of sixty-four days, a little over nine weeks, during which time the animal took no food but had access to water except while the stomach records were actually being recorded. However, in the latter part of this fast there probably entered into the work to some extent the factor of thirst, since it was found advisable not to change the animal too often from the specially constructed box as the animal at this stage of the fast had become very restless and any disturbance tended to increase the restlessness, which made the record taking very difficult. At about the beginning of the fifth or sixth week of the fast, the turtle became so restless and uneasy that a good tracing could hardly be obtained. This restlessness gradually increased in intensity until about the eighth week, then gradually began to subside until the end of the fast when the animal had become quite passive again. This indicates that the disagreeable hunger pangs in the turtle probably do not start as early as in the higher animals (45) and that it also requires a longer time to fatigue the neurones in the central nervous system which have to do with the sensation of hunger. This is the result which we would rather expect in this low form of vertebrate since periods of enforced starvation are not uncommon among wild animals. The amplitudes of the hunger contractions gradually increased with the length of the fast, the contractions became longer and the rate slower. The contractions after a nine weeks' fast (fig. 6) show an average duration of about four minutes and the intervals between the contractions vary from three and a half to four minutes. It will be seen from the above that the length of the hunger contractions under these conditions have been increased from two to

four times. It is also of interest to note of the ease with which these enormous hunger contractions are inhibited by forced respiratory movements even at the time of the onset of one of these very strong contractions as in (fig. 6, *B*). These respiratory movements or sighs are also exaggerated in prolonged fasting and during each the animal usually gives a hissing sound.

THE INFLUENCE OF TEMPERATURE ON THE GASTRIC HUNGER ACTIVITY, SEASONAL VARIATIONS AND HIBERNATION

The data collected under this head indicates that the effects of temperature on the gastric hunger mechanism of the frog must be

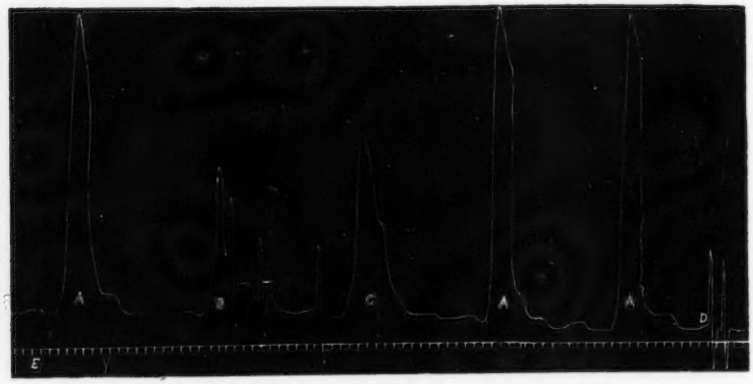

Fig. 6. Hunger contractions of the turtle's stomach after 9 weeks' fast. *A*, normal confraction. *B*, inhibition of the very strong hunger contraction by the respiratory movement, *D*, with recovery through *C*. *E* = time in 30 second intervals. Water manometer.

considered under two heads: First, the accelerating action of increased temperature on the chemical processes involved; and second, the inhibitive or retarding action of very high or very low temperatures. Biologic phenomena are undoubtedly largely the result of chemical reactions going on within the living substance, and consequently we would expect that the well-known rule of van't Hoff and Arrhenius concerning the velocity of chemical reactions in relation to temperature, should apply, that is, for each increase of 10°C. in temperature the velocity of a reaction is doubled or trebled. Thus, the above rule within certain

limits has been verified by Riddle (46) on the process of digestion in cold-blooded vertebrates, by Rogers (47) and Galeotti and Piccinini (48) on the rate of the heart beat for vertebrates and invertebrates, by De Bonis and Midulla (49) for the movements of the frog's stomach by the stomach ring method, and for many other physiological activities.

Tables 1 and 2 show the results obtained from the experiments with the number of contractions executed in the unit of time. The temperature coefficients are obtained as the quotients resulting from the formula

$$\frac{\text{Rate at } Tn}{\text{Rate at } T \times \frac{x}{10}}$$

Tn being the higher temperature, T the lower temperature, and x the difference between these two.

This is the formula used by Riddle.

TABLE 1

Raising the temperature

NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS—ROOM TEMPERATURE	NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS AFTER INCREASE OF TEMPERATURE	TEMPERATURE COEFFICIENTS
23.5° = rate of 5	32.5° = rate of 9	2.00
25° = rate of 6	32.75° = rate of 10	2.15
24.5° = rate of 6	32.25° = rate of 10	2.15

TABLE 2

Lowering the temperature

NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS—ROOM TEMPERATURE	NUMBER OF CONTRACTIONS PER 12 MINUTE INTERVALS AFTER DECREASE OF TEMPERATURE	TEMPERATURE COEFFICIENTS
20° = rate of 7	14° = rate of 5	2.33
19° = rate of 4	14° = rate of 3	2.66
21° = rate of 6.5	14° = rate of 4	2.32

An examination of the data in the foregoing tables shows that the gastric hunger contractions of the frog within certain not very wide ranges of temperature follow the rule of van't Hoff and Arrhenius, and furthermore, that the relation between the contractions and the temperature is maintained either when the animal passes from a lower to a higher temperature or vice versa. Hence, it may be concluded

that the gastric hunger contractions of the neuro-muscular apparatus is essentially dependent on chemical processes which are evolved within the stomach muscle itself. In table 2, where the temperature was diminished there appears to be an increase in the value of the coefficient as zero is approached which is entirely in harmony with the observations of Riddle (46) and Rogers (47). The observations in tables 1 and 2 were made during the month of August, 1915, and three separate animals were used for the results in each table and the findings herewith set forth were verified by control experiments.

I have also been able to carry through a sufficient number of experiments to determine the exact points at which high and low temperatures absolutely inhibit the gastric hunger contractions. The temperatures at which the hunger contractions are completely inhibited are practically constant, for both high and low temperatures and do not vary more than 0.5° in either direction in all the experiments. The average temperatures at which these contractions are completely inhibited is 35°C. for the high temperature and 13°C. for the low temperature. Lowering the temperature 0.5° or at the most 1° in the first case, or raising the temperature 0.5° or at the most 1° in the latter case starts the gastric hunger contractions again. These experiments were also performed during the month of August, 1915. The intensity of the contractions have a maximum between 15°C. and 32°C. and a minimum at lower or higher temperatures, that is, up to that limit where gastric standstill is produced. Raising the temperature also increases the strength of the contractions as well as the rate, but this effect disappears sooner than the effect of increased rate.

It is a well-known fact, that amphibians may be cooled down to −1°C. (50) and that their limbs may be frozen in ice (51), yet if the heart is not frozen these animals survive, but just how does this freezing temperature affect the gastric mechanism? Two of the animals were subjected to temperatures of 7°C. by means of the freezing mixture already described. The hunger contractions in a short time were completely inhibited and the limbs of the animals froze and became stiff and hard and when removed later from the cooling chamber and placed on the table, they rattled like stones, although their bodies were not completely frozen. Both the animals appeared lifeless except for a very slow heart beat and infrequent respirations and their eyes were closed as if asleep. One hour later at room temperature these same animals were active and began to show feeble gastric contractions which increased in intensity during the next twelve hours

but never reached the normal. These observations indicate that the gastric mechanism is capable of movement at all seasons of the year and even in hibernation when given the suitable temperature. If the temperature change is gradual, as at the rate of 10° per three hours the animal is not disturbed, but passes into a drowsy or sleepy state.

The seasonal variations as artificially produced on the frog have been verified on the turtle during the past year. Records of the gastric movements have been taken through the changes in the seasons from August, 1915, to May, 1916, inclusive. In all instances the activity of the gastric motor mechanism followed the climatic changes in temperature (fig. 7). In the coldest days of winter when the turtles showed no gastric activity or in only minor degrees, placing the animals in a tank of warm water for half an hour would greatly augment the gastric movements. However, the gastric hunger movements in winter are never as vigorous as in summer as is shown by my series of experiments. During the winter the turtles took no food although it was offered to them many times.

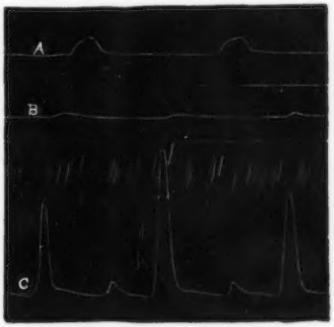

Fig. 7. Gastric hunger contractions of the turtle at different seasons of the year. *A*, contractions on a moderately cold day in winter (January). *B*, contractions on a very cold day in winter (January). *C*, contractions two and one-half days after the break of winter (March 28), weather mild and warm.

X-RAY STUDIES ON THE FROG'S STOMACH AND OBSERVATIONS ON THE EXCISED STOMACH

The general characteristics of the stomach movements in the frog have been discussed. We have now to consider the genesis of these movements. Two methods of investigation were used, namely, the X-ray and the excised stomach. The X-ray method consisted of introducing into the stomach a double walled balloon, the walls of which were separated by a thin layer of bismuth subnitrate mixed with vaseline to form a thin paste (52). The balloon was then connected with the usual recording apparatus and the fluctuations of the float in the manometer served as an indicator for studying the different

phases of the gastric movements with the X-ray. From the X-ray studies made on the frog's stomach by means of this bismuth coated balloon, the gastric hunger contractions were found to be peristaltic, the peristaltic waves originating within about 1 cm. of the cardia and then advancing rhythmically over the entire stomach toward the pyloric end increasing in strength as they proceeded. These observations on the peristaltic activity of the stomach are in agreement with the X-ray findings reported by Cannon (53) in the dog, cat, rabbit, guinea-pig and rat, and by Roux and Balthazard (54) in man, dog and frog. For the X-ray observations the frogs were placed in the box already described for the cooling experiments and this was then placed over the tube generating the rays which was properly protected. Thus by looking through a fluoroscope the gastric hunger peristalsis could be watched with the X-rays. A series of X-ray photographs was taken of the balloon in the frog's stomach during the periods of rest and at varying intervals during the period of gastric hunger contraction, the special radiographic plate being inclosed in the red envelope and carefully placed on the frog's back (figs. 8 and 9). In every case the contraction and the relaxation of the stomach corresponded precisely with the graphic record (fig. 1).

The studies on the excised stomach showed the same general phenomena as presented by the X-ray observations above. The stomach was removed with the esophagus and the intestine. The balloon was introduced into the stomach and the esophagus ligatured to the tube upon which the balloon was attached. This was connected with the recording apparatus and the stomach was placed in Ringer's solution at 30.5° C. which was found to be a most favorable temperature for gastric activity from the temperature experiments and into this solution was led a slow current of oxygen. The rate of the contractions increased but the amplitude was lessened as compared to the normal gastric movements (fig. 10). In other words, the stomach had lost its regulatory apparatus, the extrinsic nerves which govern normally the rate, size and strength of the gastric movements. These contractions from the excised stomach showed an average duration of about thirty seconds with practically no intervals of rest between the individual contractions. Increasing the pressure in the balloon does not necessarily raise the tonus, the stomach simply dilates until the limit of expansion is reached. This confirms the results on the living animal obtained from the stomach in situ which have already been described. Dropping the temperature even a few degrees reduces the size of the

Fig. 8

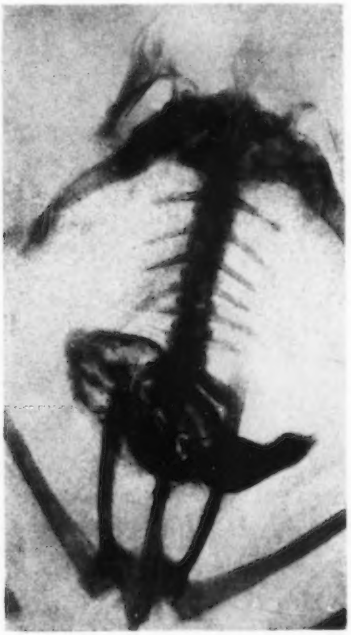

Fig. 9

Fig. 8. X-ray photograph of the bismuth balloon in the frog's stomach when the graphic record shows the stomach to be quiescent.

Fig. 9. X-ray photograph of the bismuth balloon in the frog's stomach at the height of a very strong hunger contraction.

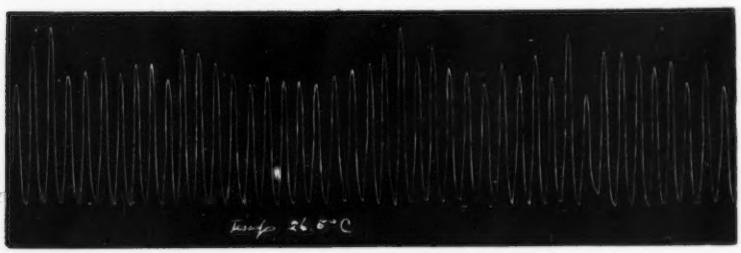

Fig. 10. Tracing from the excised stomach of the frog showing a marked tonus rhythm. Water manometer.

contractions. The curves show a distinct tonus rhythm with practically no changes in the general gastric tonus. The stomach also shows contractions with an uninflated balloon in it, as well as when it is completely empty and collapsed, but the peristaltic contractions are feeble, due to the fact that the organ is collapsed and not in a suitable condition to exhibit the full strength of the contractions. The cardia, however, is the first part of the stomach to exhibit motor activity and the last part to show it. The peristaltic waves appear to originate at the upper end of the gastric tube at a tonus ring near the cardia which forms a more or less marked depression known by X-ray workers as the "incisura cardiaca" (55), and from this point the waves sweep over the stomach toward the pyloric end as in the case described under the X-ray observations. The peristalsis observed in the frog's stomach does not always bear the character of rapidly running waves, but in certain cases may have a tonic character similar to the tonic contractions forming deep rings, thus giving the stomach the form of an hourglass but when these contractions are carefully analyzed, they are found to be peristaltic throughout. These observations are in confirmation with those of Pletneff (56) on the isolated stomachs of cats and pups with the exception that the peristaltic waves begin at the middle of the stomach rather than near the cardia, but they are contradictory to the findings of Dixon (22) and Morishima and Fujitani (29) who claim that the contractions of the stomach wall follow no strict conformable order and that the contraction rings generally show little tendency to spread, therefore according to these authors the movements of the frog's stomach are not peristaltic. However, X-ray observations by various investigators have conclusively shown the movements of the stomach to be peristaltic.

THE EFFECT OF DECEREBRATION UPON THE GASTRIC HUNGER MOVEMENTS OF THE FROG

We know, particularly through the researches of Carlson (57) on the higher animals (man and dog) that the gastric hunger contractions are inhibited by psychic stimuli and that at least in the dog they continue even after the stomach has been isolated from the central nervous system by section of the vagi and splanchnic nerves. Under these conditions stimulation of the gastric mucosa also produces the characteristic reflex inhibition but to a lesser degree than normally. It is also known that the stomach passes into a permanently hypotonic

condition after double vagotomy (33). King (40) working with decerebrate guinea-pigs has reported that the rate of the gastric contractions is increased although similar in character to those from the normal animal and also that the stomach becomes hypertonic. However, in the frog the gastric hunger contractions are not affected by the removal of the cerebral hemispheres, the graphic record of the normal and the decerebrate animal remaining practically the same (fig. 11). Since the removal of this organ produces no apparent effect on gastric motility it may be inferred that what controlling influence the brain exercises over the stomach of this animal comes from centers in the mid-brain and medulla and not from those of the cerebrum. This idea is in agreement with the more recent work of Miller (58) on dogs, who by means of unipolar stimulation in the floor of the fourth ventricle has localized in the dorsal vagus nucleus (ala cinerea) centers for cardiac inhibition and for movements of the stomach and small intestine. In the higher animals cerebral processes of pleasantness such as the appearance of a friend and unpleasantness such as fear, anger, fright, etc., reflexly inhibit the rate and tonus of the gastric movements, whereas in the case of the frog similar reflex inhibitory action on gastric motility is only exhibited to a very slight degree. This demonstrates again the simplified gastric mechanism of the frog and that the cerebral processes exert no appreciable influence on the gastro-neuro-muscular apparatus. Eight animals were decerebrated all of which confirmed the above results, even one in which the semi-circular canals were injured and all exhibited the characteristic exaggeration of the reflexes. In (fig. 11, *B*) the contractions do not appear quite as high as in the

Fig. 11. Records from the stomach of the normal and decerebrate frog. *A*, contractions from the normal stomach after three and one-half days' fast, stomach empty. *B*, contractions from the same animal's stomach twenty-one hours after decerebration. Note that the contractions in *A* and *B* are practically identical in rate and form. Water manometer.

normal animal, however, in other cases they have been found to even exceed the normal.

SUMMARY

1. The simpler gastric mechanism of the frog does not show the distinction between the digestive peristalses and the hunger contractions present in the higher animals. There is no increase in gastric tonus and the hunger contractions are practically continuous.

2. The gastric hunger movements of the turtle show a periodicity, a feature present in the higher animals. There are slight increases in gastric tonus and two types of gastric activity are exhibited.

3. The hunger contractions in both the frog and turtle are inhibited or weakened by the introduction into the stomach of small quantities of water, weak alkali and acid, but the inhibitory effects are not produced as quickly as in the higher animals. When these substances are introduced into the mouth of the frog the inhibitory effects are very slight.

4. Water, weak alkali and acid when introduced into the filled stomach of the frog inhibit the digestive peristalsis in the same degree as they inhibit the hunger contractions of the empty stomach. In the higher animals normal digestive peristalsis is not inhibited by these substances in the same amounts.

5. During prolonged fasting in both animals the hunger movements are greatly augmented and the contractions show a marked increase in the amplitude directly proportional to the length of the fast, but no increase in gastric tonus. In the higher animals like the dog there is a marked increase in the gastric tonus in prolonged fasting, and this increase is inversely proportional to the decrease in the amplitudes of the hunger contractions.

6. Within certain not very wide ranges of temperature the hunger movements of the frog's stomach fall within the limits of van't Hoff's rule.

7. The hunger contractions of the frog's stomach are completely inhibited at 13°C. for low temperature and at 35°C. for high temperature. Raising the first temperature or lowering the second 0.5° or at the most 1° starts the hunger contractions again.

8. In both animals the gastric hunger activity is proportional to the climatic changes in temperature within certain limits as is indicated by van't Hoff's rule.

9. The gastric hunger movements are peristaltic and the waves start near the cardia at the "incisura cardiaca."

10. The stomach of the decerebrate animal behaves in the same manner as that of the normal frog.

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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

I. Disturbances of A-V Conduction

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INTRODUCTION

In a former paper (1) a functional differentiation in the A-V funnel of the lizard and turtle was demonstrated, in that the right and left parts of this funnel when reduced to a very narrow bridge could, either of them, conduct the contracting impulse from the A to the V, so that there was coördination between them. Furthermore it was found that if one of the parts of the connection were alone cut, there resulted a severe and long lasting block, while if both were cut permanent dissociation between A and V followed. In two later papers (2 and 3) the anatomical grounds for this physiological differentiation were shown to exist in a more intimate connection between the right and left funnel fibers and those of the inner wall of the ventricle than at other parts of the funnel.

After the publication of my first paper two others dealing with this same general subject appeared. Nakano (4) showed that the dorsal, right and left parts of the A-V funnel of the frog and salamander possessed a higher degree of conductivity than the remaining parts. And Mangold and Kato (5), from an analysis of the effects on the electrocardiogram of cutting and piercing the A-V connection in the fowl, have traced the pathways of the contracting impulse, which begin in the "sinus node" (6) and are collected at the level of the A-V boundary into a bundle, the principal branch of which goes into the right ventricle along the septum, while the other goes through the septum to the left and connects with the papillary muscles. Mangold (7) has given a summary of all these results.

To carry on the investigation of this problem of functional differentiation in the A-V connection a physiological and anatomical study of

this region in the alligator's heart has been undertaken, the results of which will be published shortly.

Wishing to continue the investigation of this differentiation in the turtle heart it was necessary to repeat the earlier experiments that had been carried out on the heart of the lizard and of the turtle (Clemmys lutaria) on a native species. Malacoclemmys geographica was the species used.

EXPERIMENTAL

The methods of experimentation can be briefly described. The cervical cord was cut through and the brain and spinal cord destroyed. The plastron was then removed as rapidly and with as little loss of blood as possible and the heart laid free by slitting up the pericardium. Before and after opening the pericardium the frequency of beat was taken with a stop-watch. The heart was then suspended from the right auricle and the apex of the ventricle. In some cases the heart was excised, in others left in situ and a record of the "normal" heart taken for frequency of beat and length of A-V interval. To determine the parts of the funnel of greatest conductivity portions of this were cut through with a pair of fine scissors, one of two procedures in the manner of cutting being followed. Either a series of small cuts was made and after each a record taken to observe the effect of the cut on A-V conduction, and the cutting thus continued until an interference with the coördination of A and V took place, which was then allowed the opportunity of recovering, after which the process was repeated. Or a large portion of the funnel down to a desired narrow bridge of tissue was cut through at once, and a record taken to observe the resultant degree of block and its recovery.

It must at first be pointed out with regard to the study of the A-V conduction time, etc. that, as has been earlier noticed (1 and 4), every mechanical disturbance of the heart has some effect on its activity, which may express itself in a retardation or acceleration, or a short standstill of both A and V or of V alone, extra systoles, group formation, pulsus alternans, ventricular fibrillation, etc., all of which are of short duration and are not to be regarded as typical effects of the particular operation. Lewis (8) noted that once the pericardium is opened irregularities in the beat occur which he considers due to changes in temperature and in the position of the animal. Meek and Eyster (9) also note that handling the heart leads to irregularities.

RESULTS

Briefly stated, the results obtained by cutting through parts of the funnel of Malacoclemmys substantiate in the main those obtained with Clemmys. Following the plan of Nakano (4) attempts were made to divide the ring into eight parts, but it was soon found that by this nothing was to be gained. Particular attention was also paid to the dorsal portion of the connection, since Nakano had found that in the frog this was an important part, but with negative results.

The right and left parts of the funnel were again found to be possessed of a higher degree of conductivity than the other parts. But my former results with regard to the consequent marked and long lasting block when the one or the other of these parts was cut through, and the permanent dissociation produced when both were severed have not been obtained. For in Malacoclemmys the broad ventral and dorsal portions of the funnel remaining when the right and left sides are cut through are fully capable of conducting the contracting impulse so that the ventricle, after longer or shorter standstill, beats again, either in a low degree of partial block which shortly recovers, or immediately coördinated in a 1 : 1 rhythm. In other words no part of the connection in Malacoclemmys can be said to be indispensable for the coördinated conduction of the impulse to the V. But nevertheless, as in the heart of Clemmys, the right and left parts of the funnel are possessed of a higher degree of conductivity. For along either of them when all other parts are severed, and when they are reduced to a mere thread of tissue incapable of being further narrowed, the impulse from the A is able to reach the V and cause it to beat coördinated with it and in a normal 1 : 1 rhythm.

The cutting of any part of the connection increases the length of the A-V interval to a greater or less extent, but with apparently no reference to the portion severed (see table 1). In contrast to the relatively slight effects on lengthening the A-V interval when a particular portion or portions of the funnel are severed are those when all but a certain small part is cut through. Looking over these examples it will be apparent that in the heart of Malacoclemmys, as in Clemmys, the right and left parts of the funnel are the most functional, not only from the standpoint of it being possible to narrow them down more than the dorsal and ventral portions without affecting coördinated conduction or obliterating it entirely, but also from the standpoint that when narrowed down to so small a bridge of tissue that another cut

TABLE 1

Effects of cutting parts of the A-V connection on the length of the A-V interval

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
7	19.5	10.35		1.12		18	
		10.38	Right and left, leaving broad ventral and dorsal connection	1.20	0.08	17	Short standstill of V, followed by 1 : 1
8	18.2	9.20		0.97		18	
		9.26	Right and left deeply	1.41	0.44	18	Short standstill of V, followed by 2 : 1, quickly 1 : 1
		9.28		1.31	0.34	17	
10	20.0	10.56		0.72		22	
		10.59	Right and left deeply	1.62	0.90	20	Short standstill of V, followed by 1 : 1
11	22.8	8.57		0.83		24	
		9.00	Right and left lightly	0.95	0.12	24	
		9.02	Right and left deeply	1.03	0.20	24	Short standstill of V, followed by 1 : 1
2	22.0	9.45		0.96		18	
		10.00	Right	0.96	0.00	18	
3	22.0	9.25		0.89		18	
		9.28	Left	0.94	0.05	17	
24	22.0	10.00		0.86		18	
		10.06	Left	0.94	0.08	18	
25	21.0	9.35		0.79		19	
		9.40	Dorsal	0.87	0.08	18	
5	21.2	10.24		1.04		18	
		10.28		1.08		16	
		10.30	All except left				Partial block, recovery to 1 : 1
		10.50		1.62	0.54	17	
3	22.0	9.20		0.88		18	
		9.25		0.89		17	
		9.28	Left	0.94	0.05	17	
		9.30	All except narrow bridge on right	1.62	0.68	17	Short partial block, followed by 1 : 1

TABLE 1—Continued

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
6	18.3	9.20		0.95		21	
		9.23 to 9.44	A series of cuts leaving bridge on dorsal	1.88	0.93	20	
		9.45		1.93		20	At 9.46 irregular dropping of V systoles, ending in dissociation
12	20.9	9.22		0.99		24	
		9.26	Right and left lightly	1.08	0.09	24	
		9.28 to 9.33	All remaining except dorsal	1.60	0.72	24	Fibrillation of V, followed by 1 : 1
7	19.5	10.35		1.12		18	
		10.38	Right and left lightly	1.20	0.08	17	
		10.42	Dorsal, leaving ventral			18	Standstill of V for three minutes, then 4 : 1, 2 : 1 and at 10.46 1 : 1
		10.46		1.32	0.20	18	
		10.50	Ventral bridge narrowed			18	Permanent block

would necessarily sever it the A-V interval is not lengthened so much on the average as when the dorsal or ventral portion of the funnel alone remains.

It is clear that whether and to what degree a retardation of the A-V interval will take place depends not merely upon general injury, such as the length of the experiment and the number and extent of the cuts made, but also upon the importance for A-V impulse conduction of the part injured. The width of the bridge of tissue is less important for coördinated conduction than the part that has been left. There is an independence also between the ability to conduct and the velocity of conduction. For whenever a partial block has been produced which later disappears the A-V interval is longer than it was before the block occurred.

Gaskell (10) from a few experiments, which were far from complete or conclusive, on cutting away various parts of the A-V connection, stated that the right ventral portion of the connection just under the aorta ("the upper portion of the right ring") was the most important part in the conduction of the contracting impulse from A to V. (Although there is no connection here between the musculature of the A and V, since the funnel musculature goes over into that of the bulbus (2 and 3)). Meek and Eyster (9) have found that the impulse in going from the A to the V reaches the various parts of the ring in the following order: anterior part, left part, right part. They call attention to the similarity between their results and those of Gaskell, and also believe that they have substantiated the old belief that the contracting impulse is of the nature of a wave which sweeps over the sinus and auricles and spreads downward from the circular fibers of the A-V ring over the V to terminate at the apex. Negativity in the basal portions coming first is supposed to be largely determined by proximity to the ring, although they do not exclude the possibility of there being certain pathways along which the wave may pass more directly or with greater ease, as shown by their results on the earlier negativity of the left part.

Somewhat opposed to these results are those of Lewis (8) obtained with the heart of Testudo graeca. According to him the distribution of the excitation process over the ventral surface of the V is that, as a rule, the base and central region, or the central, is earliest and the apex the latest. Sometimes he obtained a high reading from the region of the base in the neighborhood of the truncus arteriosus and sometimes the right margin was found to be activated at a later time than the left. The lateral and posterior surfaces of the heart showed the same distribution. From a comparison of readings taken from the inner surface of the V and compared with those from the outer, Lewis comes to the conclusion that there is in the V no special tissue which conducts more rapidly than the surface muscle fiber, as he found also to be the case in the heart of the toad. It is interesting to note that he obtained, table 4, p. 233, in the toad the earliest internal reading near the ring in front at the left auricular border, and that on p. 243 he makes the statement that the earliest internal readings are taken from the portion of the lining which borders the A-V ring, though he does not state just what part of the ring is the earliest. It remains to consider Lewis' idea of the manner in which the impulse from the auricles is communicated to the V (see his fig. 6, p. 236). He regards

it not as in the nature of a simple surface spread, but, as in the V of the dog, a distribution from within outwards from the ring to the surface through the wall of the heart. "The musculature of the A and V do not meet at the surface of the A-V line but the ring is prolonged as a tube for some distance into the V; passing downwards it meets and fuses with the processes of muscle which radiate towards the central surface zone and apex of the heart. The trabeculae first carry the excitation to the central surface zone of the heart. Later the wave courses farther toward the apex and at the same time mounts to the base."

The results of these investigators are given to point out the want of agreement which exists between them, and how they both differ from what has been found by the method of severing portions of the A-V funnel. Perhaps a word more may be added regarding the arrangement of the fibers of the funnel. In the first place the physiological differentiation in the right and left portions of the funnel has been explained, (2 and 3), by the closer connection here. On the other hand the ventral and dorsal parts of the funnel are the first to come into union with the fibers of the ventricle and we might therefore expect these portions of the ventricle to become negative earlier. But Lewis finds that the same thing occurs on the lateral surface of the ventricle, so that it may be said that the places of earliest negativity are where the funnel musculature fuses with that of the ventricle. From Lewis' diagram and tables it can be seen that the heart of the tortoise differs from that of the toad in that the excitation wave reaches the basal portion of the surface of the heart of the tortoise before it reaches the apex, while the surface basal muscle of the toad heart is the last to be activated. This is in all probability due to the reason which Lewis (p. 246) gives for this chief difference between the distribution of the excitation process in the toad's heart and in the tortoise heart, namely, that in the tortoise ventricle the fusion of the ring musculature with the ventricular wall takes place at a level relatively nearer to the base than in the toad's heart.

It would be completing a detail of this phase of the problem of A-V conduction to study tracings of the passage of the wave of negativity before and after cuts of the kind above described were made. This would undoubtedly bring out more clearly the effects of the severing of this or that part of the funnel and the effects on the velocity of conduction between A and V.

DISTURBANCES OF A-V CONDUCTION

The disturbances of A-V conduction which are brought about by means of cutting the connection are of considerable general interest.

As earlier stated with every cut made through a part of the A-V funnel there is, as a rule, an increase in the length of the A-V interval, without necessarily producing a condition of block (see table 2). This increase either becomes spontaneously greater or remains constant, and even in a few cases with the improvement in conduction decreases.

LENGTHENING OF A-V INTERVAL LEADING TO PARTIAL BLOCK

Of much greater interest than the mere slowing of conduction between A and V are those cases in which the retardation leads to the production of block (partial). The various degrees of such disturbances can be observed with great clearness in the turtle heart. A short description accompanied by curves and tables will now be given of them.

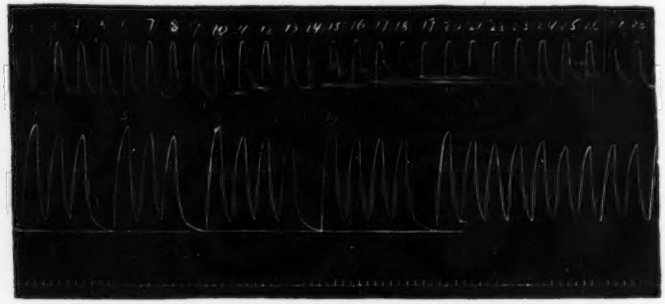

Fig. 1. Partial block characterized by gradual increase in length of A-V interval.

As is well known one of the types of partial block is characterized by a gradual increase, with every heart cycle, of the A-V interval until a contraction of the V is missed (11 and 12). During the pause the V, or the connecting system, recovers and the next cycle has a shorter A-V interval, which with every successive cycle again increases until another V systole is missed and thus the process is repeated. In figure 1 an example of such a type of block is shown and its final recovery to a normal 1 : 1 rhythm. The lengths of the A-V intervals are given in table 3 under No. 18 a. It will be observed that at the end where

TABLE 2

Effects of continued cutting of the A-V connection on the length of the A-V interval

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
3	9.20		0.88	18	
	9.25		0.89	17	
	9.28	Dorsal ligament and small cut on left	0.94	17	
	9.30	All except a bridge on right	1.62	17	
	9.40		1.60	16	
	9.50	Right bridge narrowed	1.71	16	Rhythm = 2 : 1
	9.53		2.09	16	Rhythm = 1 : 1
	9.58	Right bridge narrowed		16	Standstill of V for 2 minutes, followed by 3 : 1, recovery to 2 : 1
	10.26		1.77	15	Rhythm = 2 : 1
	11.20		1.79	12	Rhythm = 1 : 1
6	9.20		0.95	21	
	9.23	Right	1.06	22	
	9.25	Left	1.31	21	
	9.29	Right and left more deeply on dorsal	1.51	20	
	9.35	Ventral	1.74	20	
	9.41	Septum	1.76	20	
	9.44	Dorsal slightly narrowed	1.88	20	
	9.45		1.93	20	
	9.46			20	Block, and finally dissociation
8	9.20		0.97	18	
	9.26	Right and left deeply	1.41	18	Short standstill of V, 2:1 rhythm of short duration, 1 : 1
	9.28		1.31	17	
	9.30	Ventral entirely through	1.71	16	
	9.34		1.60	16	
	9.38	Dorsal slightly from right	1.71	16	
	9.44	Dorsal slightly from right	1.62	15	Rhythm = 2 : 1
	9.45		1.91	15	Rhythm = 1 : 1
	9.50		1.71	15	
	10.09		1.77	15	
	10.11	Dorsal narrowed from left		15	Standstill of V, later an independent V rhythm

TABLE 2—Continued

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
10	10.56		0.72	22	
	10.59	Right and left deeply	1.62	20	Short standstill of V, followed by 1 : 1
	11.03	Dorsal	1.39	21	Rhythm = 4: 1
	11.06		1.39	22	Rhythm = 2: 1
	11.07		1.85	22	Rhythm = 1: 1
	11.15		1.59	22	Rhythm = 1: 1
	11.18	Ventral narrowed		22	V is still
11	8.57		0.83	24	
	9.00	Right and left lightly	0.95	24	
	9.02	Right and left deeper	1.03	24	Short standstill of V, followed by 1: 1
	9.04	Dorsal and ventral from left		24	Dissociation
	9.07		1.48	24	Rhythm = 1: 1
12	9.22		0.99	24	
	9.26	Right and left lightly	1.08	24	
	9.28	Right and left deeper	1.36	24	
	9.32	Ventral		24	Fibrillation of V, followed by 1 : 1 after a single large V_s
	9.33		1.60	24	

the block disappears the A-V interval has a length almost equal to that after which the V systole was in the habit of falling out. In all figures the upper tracing is of the contractions of the right auricle, the middle those of the ventricle. The bottom line is time in seconds.

Figure 2 illustrates the influence of A rate and irregularities in A beat in the formation of this type of block. A block between A and V had been caused at 9.54 by cutting through a part of the funnel. The remaining funnel fibers gradually recovered from the injury, the V beginning by giving single, isolated contractions at 9.55, and at 9.57 suddenly beginning to beat in a 4 : 1 rhythm. At 9.59 this became 2 : 1 and continued regularly so until 10.04 at which time the record shown was taken. The A and V are for the most part beating in a 2 : 1 ratio, but occasionally, for several successive contractions, the rhythm is 1 : 1. When the lengths of the A-V intervals are measured (see table 4) under 10.04 it is seen that they are of a constant value, 0.86 seconds (0.8 second has been added since the A lever is that much

TABLE 3

Lengths of A-V interval in cases of partial block. Note the increase in length of interval when the rhythm becomes 1 : 1

NO. 18 A		NO. 10		NO. 11		NO. 20		NO. 18 B		NO. 4		NO. 14	
A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V
1	1.68	1	1.31	1	1.39	1	1.46	1	1.79	1	1.45	1	0.99
2	1.84	2		2		2		2	2.02	2		2	
3	2.01	3	1.31	3	1.39	3	1.46	3		3	1.45	3	0.99
4		4		4		4		4	1.79	4		4	
5	1.68	5	1.31	5	1.39	5	1.46	5	2.02	5	1.08	5	0.99
6	1.90	6		6		6		6		6		6	1.20
7	1.96	7	1.31	7	1.39	7	1.46	7	1.79	7	1.22	7	
8		8	1.85	8	1.46	8		8	2.02	8	1.43	8	0.99
9	1.62	9	1.62	9	1.51	9	1.46	9	2.02	9	1.51	9	1.04
10	1.79	10	1.40	10	1.40	10	1.86	10		10	1.62	10	1.12
11	1.96	11	1.40	11	1.40	11	1.84			11		11	1.22
12	2.12	12	1.40	12	1.39	12	1.80			12	1.36	12	1.28
13						13	1.77			13	1.43	13	1.28
14	1.68					14	1.77			14	1.48	14	
15	1.84					15	1.68			15	1.52	15	1.04
16	1.96					16	1.74			16	1.48	16	1.16
17	2.12					17	1.68			17	1.52	17	1.28
18						18	1.68					18	1.28
19	1.74					19	1.71					19	1.16
20	1.96					20	1.68					20	1.16
21	1.96					21	1.71					21	1.16
22	1.96					22	1.68						
23	1.96					23	1.71						
24	1.96					24	1.68						
						25	1.65						
						26	1.60						
						27	1.60						

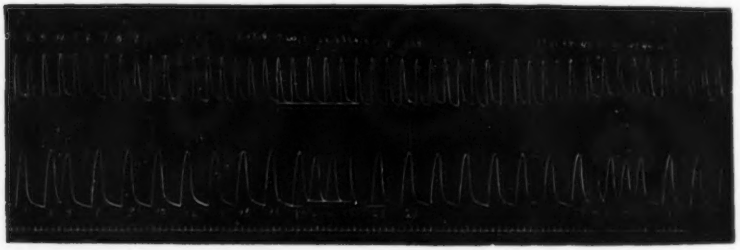

Fig. 2. Influence of A rate on partial block.

TABLE 4

Influence of A rate on formation of partial block (cf. figs. 2, 3 and 4)

10.04			10.10			10.22		
A_S	A-V	A-A	A_S	A-V	A-A	A_S	A-V	A-A
1	0.79		1	0.97		1	1.16	
		2.08			2.43			2.49
2			2	0.97		2	1.16	
		2.38			2.43			2.66
3	0.86		3	0.97		3	1.08	
		2.52			2.35			2.99
4	0.86		4			4	1.16	
		2.30			2.43			2.82
5			5	0.97		5	1.08	
		2.38			2.27			2.66
6	0.86		6			6	1.16	
		2.30			2.27			2.57
7			7	0.97		7	1.02	
		2.38			2.43			2.49
8	0.86		8			8	1.08	
		2.38			2.35			2.49
9			9	0.97		9	1.16	
		2.38			2.35			2.57
10	0.86		10			10	1.16	
		2.16			2.43			2.91
11			11	0.97		11	1.02	
		2.30			2.99			2.91
12	0.86		12	0.97		12	1.02	
		2.16			2.75			2.66
13			13	0.97		13	1.02	
		2.23			2.59			2.49
14	0.86		14	0.97		14	1.02	
		2.16			2.43			2.41
15			15	0.97		15	1.02	
		2.16			2.51			2.41
16	0.86		16	0.97		16	1.02	
		2.16			2.51			2.91
17			17	0.97		17	1.02	
		2.23			2.48			2.99
			18	0.97		18	1.02	
18	0.86				2.43			2.74
		2.16	19			19	1.02	
19					2.43			2.49
		2.16	20	0.90		20	1.02	
20	0.86				2.43			2.49
		2.66	21	0.97		21	1.02	
					2.51			2.49

TABLE 4—Continued

10.04			10.10			10.22		
A_s	A-V	A-A	A_s	A-V	A-A	A_s	A-V	A-A
21	0.86		22	0.95		22	1.02	
		2.66			2.43			2.41
22	0.86		23			23	1.02	
		2.52			2.43			2.99
23	0.86		24	0.90		24	1.02	
		2.38			2.51			2.66
24			25	0.95		25	1.02	
		2.30			2.43			
25	0.86		26					
		2.23			2.43			
26			27	0.90				
		2.30			3.08			
27	0.86		28	0.95				
		2.16			3.08			
28			29	0.95				
					2.59			
			30	0.95				
					2.67			
37	0.86		31	0.95				
		2.08			2.59			
38			32	0.95				
		2.16			2.59			
39	0.86		33	0.95				
		2.66						
40								
		2.52						
41	0.86							
		2.38						
42								
		2.38						
43								
		2.30						
44								

too far to the right). The A-A interval is, however, variable and shows a gradual decrease in length after A pauses which occur more or less regularly. These A pauses, or sudden lengthenings of the A-A interval allow the A-V connection (or the V) time to recover between the A systoles so that the impulse reaches the V. Perhaps too the strength of the A impulse is greatest after the pause and decreases gradually

as the rate increases. After the pause the rate of beat increases as evidenced by the gradual shortening of the A-A interval until finally the A-V rhythm becomes 2 : 1, the A-V connection being able to conduct only every other impulse, or the V to respond to only every other.

In figure 3 a later record of the same heart is shown taken at 10.10 (see table 4). The block has improved so that the rhythm is for the most part 1 : 1. The A-V interval has increased somewhat being now 0.97 second and constant (0.7 second has been added). The rate of A has decreased, being now about 25 per minute. Here the same tend-

Fig. 3. Same as figure 2.

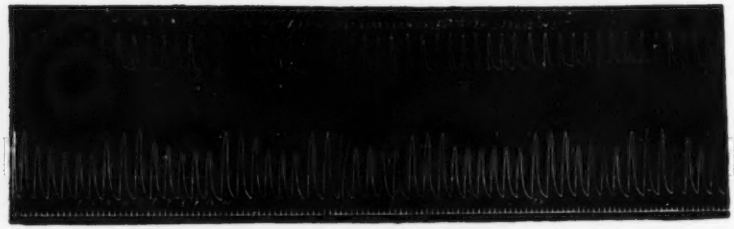

Fig. 4. A further influence of A rate on the character of V beat.

ency on the part of the rate to gradually increase is manifest so that the rhythm either becomes 2 : 1 or a V systole is missed. Again the pause, lengthening the A-A interval, lets the impulse through every time and the rhythm becomes 1 : 1 to remain so until the rate of A becomes too rapid.

The influence of auricular pauses is still further shown in figure 4 which is a record of the same heart taken at 10.22 (table 4). The A-V interval (0.7 second added) is practically constant, showing only slight variations which, however, do not give evidence of having any influence on the V contractions. The frequency of A beat is now about

23. Every time the A pauses, to beat again with a lengthened A-A interval which gradually decreases, the following three V systoles are larger, after which the A rate becoming more rapid the strength of the impulse getting through the A-V connection is diminished and fewer fibers of the V are excited to contract and, therefore, the V systole is not so vigorous.

It is possible to obtain all grades of this type of partial block, though the most common cases are those in which every second, third or fourth V systole falls out. Many cases of the other type of partial block were also observed and tracings made showing the disappearance of the disturbance to conduction. Several points have been noted which

Fig. 5. Recovery of a 4 : 1 rhythm to a 2 : 1.

seem of interest. In figure 5 is shown an example of the transition of a 4 : 1 to a 2 : 1 rhythm, the values of the A-V interval being 1.33 seconds before and after the transition (4.0 seconds subtracted). In figure 6 is shown the final transition to 1 : 1 (see table 3, No. 10, 3.6 to 4.0 seconds subtracted). Note that the A-V interval is increased at the time of transition. In the next column (No. 11) are given the values of a case where the A-V interval though increased at the time of transition quickly returns to its previous value. No. 20 represents another case with a marked increase in length of A-V interval as the block disappears. This at first decreases and later develops an alternation of a slightly longer and a shorter A-V interval, and a corresponding higher and lower V contraction or pulsus alternans. It is to be

noted in all that at the moment of transition there is a greater or less increase in the length of the A-V interval, which may later disappear, or decrease more or less.

Sometimes the transition from a 4 : 1 rhythm takes place to a 3 : 1 (instead of to a 2 : 1) which eventually changes to a 2 : 1 rhythm.

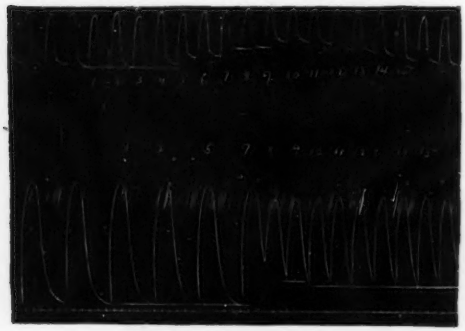

Fig. 6. Recovery of a 2 : 1 rhythm to a normal 1 : 1.

A characteristic of all cases which have been registered of a 3 : 1 rhythm changing to 2 : 1 consists in the apparent inability of the reduced (injured) A-V connection to allow the increased strength of impulse to get through once and for all. There is always a return for one or more cycles to a 3 : 1 ratio after which the 2 : 1 rhythm is permanently attained.

Figure 7 shows a very brief instance of this sort of thing. The V had for some time been contracting after every third A systole. Suddenly after A_8 9, the V contracted thus missing but one A beat instead of two. After that it again missed two beats and then followed regularly after every second A systole. Sometimes it takes very much longer for the 2 : 1 rhythm to be attained and the alternation between it and the 3 : 1 rhythm lasts for some time.

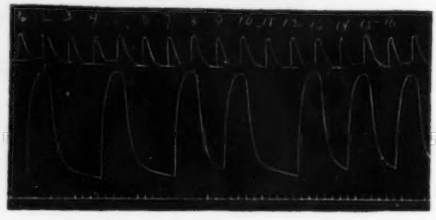

Fig. 7. Recovery of a 3 : 1 rhythm to a 2 : 1, showing the inability to make the change once and for all.

A block represented by a 2 : 1 rhythm may change, in addition to the method illustrated in figure 6, by passing through an intervening stage, characterized by the gradual increase in the length of the A-V interval (see fig. 8). At 9.37 the A and V were beating regularly in a 2 : 1 rhythm. Unfortunately the drum had to be stopped and before it was again started it was observed that not every second

V systole was being dropped, but every third, in other words the rhythm was now 3 : 2, as shown in the second part of figure 8. The values of the A-V intervals are given in table 3, under No. 18 b (0.4 second subtracted). This has become therefore a typical case of a gradual increase in length of A-V interval to a maximum after which a V systole is dropped. The records in these two figures are early stages

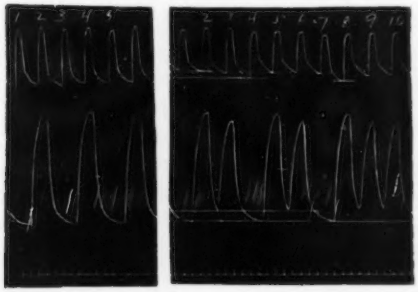

Fig. 8. A stage in the recovery of a 2 : 1 rhythm, showing intermediate conditions of 3 : 2 and 4 : 3 rhythm. Cf. Fig. 1.

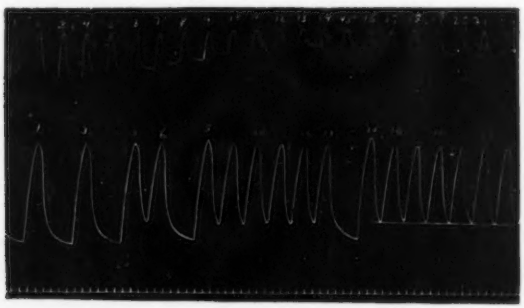

Fig. 9. Recovery of a 2 : 1 rhythm to a 1 : 1, passing through intermediate tages of gradually increasing length of A-V interval.

of the block illustrated in figure 1 (see table 3, 18 a). The ratio of A and V quickly became 4 : 3. At 9.40 (fig. 1) it is 5 : 4 and soon after this the block disappeared, and coördination took its place.

In No. 4 (table 3) the values of the A-V intervals of a much shorter case are shown, and in figure 9 another case is illustrated (see table 3, No. 14, 1.7 seconds subtracted). These cases are of particular in-

terest in that they point to the view that these two types of block are closely related and that the type in which there is a gradual increase in the length of the A-V interval is intermediate between 2 : 1 block and coördinated rhythm, although a 2 : 1 block often disappears without passing through this intervening stage.

The results of these experiments are not entirely in accord with the view expressed by Gaskell to the effect that the degree of block is directly proportional to the thickness of the connecting bridge left between the A and the V, and that by gradually decreasing this, a higher degree of block is to be produced. As pointed out in the first portion of this paper, rather more emphasis is to be placed on the particular part of the connection left, since some parts of the funnel are more effective than others in conducting the contracting impulse. Partial block can of course be produced in the way in which Gaskell claims but only within limits. For if time is allowed it is nearly always seen that the remaining bridge is more functional than at first seemed to be the case, in that the degree of block decreases and finally disappears, the right and left parts of the connection having the greatest capacity for recovery.

VENTRICULAR RHYTHM

In Clemmys complete block was always to be obtained by severing the most important parts of the A-V funnel, namely, the right and left. In Malacoclemmys, as has been pointed out above, this is not the case. But complete block can very readily be obtained in this form by narrowing down the connection left after the right and left parts are cut through. This complete block may consist in the V becoming quiescent and remaining so as long as it was observed. Usually, however, the V after an interval begins to beat, at first slowly and gradually increasing in rate, but entirely independently of the A. The rate of the independent V rhythm has never been seen to approach that of the sinus rhythm, as was observed in the heart of Clemmys and figured in my earlier paper on pp. 199 ff., where in old hearts the V sometimes initiated the rhythm of the heart, and beat more rapidly than the A. (Some of these figures perhaps represent cases of very much lengthened A-V, rather than V-A). The rhythm producing power of the V, or rather of the A-V funnel, of Malacoclemmys does not seem therefore to be so highly developed as in Clemmys. But it can institute a rhythm as was shown to be the case in the lizard ((1) p.

168, fig. 33), where after the connection between A and V had been completely cut through, the V continued to beat and in the ratio of 10 contractions to 25 of the A. In one case of the development of a funnel rhythm a severe block was produced at 9.34 from which the connection gradually recovered letting through impulses so that the V gave isolated contractions which at 9.39 were at the rate of about 1 to 7 A systoles. At 10.10 and 10.20 the connection had much improved for the V was beating once to every three A beats and apparently coördinated. But a cut at 10.29 completely severing the bridge had no other effect than to slightly retard the V, thus showing the

Fig. 10

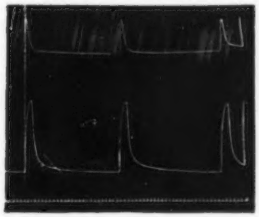

Fig. 11

Fig. 10. Independent A and V contractions after the A-V connection had been severed.

Fig. 11. An example of an A-V funnel rhythm.

independent origin of the V rhythm. Figure 10 is a record of the heart beat after the A-V connection had been severed.

To remove the possibility of this independent V rhythm being due to the Na in the Ringer solution with which the hearts were moistened a number of experiments were made in which the heart was placed in a chamber kept moist by filter paper saturated with tap-water. The results of these experiments are summarized in table 5. Several records were taken showing the rhythm originated in the A-V funnel after the sinus had been removed, and one of these is shown in figure 11. As well known, Engelmann (13) demonstrated a V-A rhythm in the frog after standstill following clamping off the sinus.

TABLE 5

Instances of the development of independent V rhythm

NO.	TEMPERATURE	FREQUENCY	TIME	OPERATION	RESULTS
52	20.5	22	2.15	First and second Stannius ligature	
		15	5.30		A is still, 1 V_s every 25–35 seconds
53	18.0	18	10.05	First and second Stannius ligature	
		17	12.20		A is still, 1 V_s every 40, 20, 35, 20, 35, 20, 35, 19, 24, 33 etc. seconds
54	18.4	21	10.30	First Stannius ligature	
		18	11.44		V and A beating about 3 per min.
			11.46		1 V-A cycle every 30, 27, 30, 35, 34, etc. seconds.
			12.45		1 V-A cycle every 30, 29, 29, 33 50, 22, 24, etc. seconds.
55	19.4	24	11.00	First Stannius ligature and V trimmed down so that only a small portion surrounding funnel remains	
		26	11.10		1 cycle every 10, 40, 27, 20, 23, 15, 27 etc. seconds.

DISCUSSION

A functional differentiation exists in the A-V funnel of Malacoclemmys geographica as in Clemmys lutaria. The right and left parts of the funnel possess in a higher degree than other parts the ability, when the one or the other is the sole connection between A and V and when reduced to a very narrow bridge, of conducting the A impulse so that A-V rhythm is coördinated. The production of severe and long lasting block when one or the other of these two parts is cut through, and the complete dissociation produced when both are cut through which was obtained with Clemmys and Lacerta have not been substantiated

with this heart, and no part of the funnel can be said to be indispensable for coördinated conduction.

It will facilitate the discussion of the evidence regarding block given by the above results to briefly review the opinions of others concerning this phenomenon. Engelmann (14) explains the type of partial block, in which a V systole more or less regularly drops out after the gradually increasing A-V interval reaches a maximum, by the effect of the V systole on the length of the A-V interval. He assumes a summation of the retarding influence of the successive stimuli so that the A-V interval becomes larger and larger up to a maximum which is retained, or at which from greater fatigue a V systole is dropped. During the resting period the conductivity of the A-V connection is improved and the V contracts after the next A systole with a shorter interval or latent period, after the next A systole with a slightly longer interval and so on until a V systole is again dropped, and the process thus continued. Later (15) he concludes that the gradual increase of the latent period for excitation of the V plays a part in the falling out of V systoles which occur after the maximum value of the A-V interval has been reached, this increase in latent period pointing to a decrease in the velocity of conduction.

Muskens (16) who observed this type of block after vagal stimulation claims that it is due to blocking of the wave of excitation so that it does not reach the V, and in poorly nourished hearts, to the increased inability of the V to conduct after every V systole.

Hering (17) points out that there is no proof, excluding those cases in which the connecting fibers have been directly injured, that the dropping out of the V systoles is due to a temporary loss of conductivity on the part of the A-V connection fibers, but that the explanation is to be found in the loss of irritability of the V, in the lengthening of the refractory period (see also Straub, 18). He admits however that the gradual increase in the length of the A-V interval can indirectly cause the dropping out of a V systole by the lengthening of the refractory period of the V, but although he grants the possibility in certain cases of the failure of the A-V connection fibers to conduct causing the dropping out of a V systole, he sees nothing to induce him to accept the view. In a later publication (12) he refers the falling out of a V systole after a gradually increasing A-V interval to a retardation in A-V conduction which takes place in Tawara's node.

Von Kries (19) considers this type of block, occasional cases of which he obtained when the contractions of the V were slowed by cooling it,

as due to the V systoles being shoved a little further and further apart until an auricular impulse falls into the refractory period of the preceding V systole, which results in a V systole being missed. After the rest the next V systole follows the A systole after a shorter interval and the process begins again. The impulses going from A to V must be assumed not to be momentarily concentrated but spread over a certain short period of time. Furthermore he points out the necessity of assuming in the heart muscle a refractory period for conduction after a stimulation as well as for contraction. It is interesting to note in von Kries' curve on p. 488 that the A systole corresponding to the V systole which is missed is lower than the other A systoles. Perhaps we have here an example of periodically weakened A impulses.

The other type of partial block is also thought by Engelmann (15) to be due to a retardation of conduction by which he explains rhythmic disturbances of the heart such as hemisystole and pulsus alternans. If a stimulus comes to the heart muscle at the moment when the ability to conduct has not returned to normal, then a partial contraction of the V musculature will result, and only later when all the fibers have regained their normal ability to conduct will the V as a whole take part in the systole. Differences in conductivity are not claimed to be always the explanation of such disturbances, and he admits that differences in contractility of the muscle fibers, temporarily weakened by contraction, are also important factors.

Erlanger (20) from experiments on compression of the bundle in the dog and on strips of turtle heart concludes that it is the strength of the impulse which determines whether or not it will, after having passed a partial block, serve as an efficient stimulus to the part beyond. He found it impossible to explain partial block by assuming an increase in the refractory period at the seat of injury. Furthermore, he accounts for the development of partial or complete block when the auricular rate is increased in part by the diminution in strength of the A impulses, and in part by the fact that more of the impulses fall into the ventricles before their irritability reaches the level at which the impulses of the normally beating auricles would have become efficient stimuli.

Hering (21) brings into line with his explanation of pulsus alternans this type of partial block. Pulsus alternans he considers due to the lengthening of the refractory phase of the V fibers which therefore do not respond to the stimulus. The periodic dropping out of a V systole is to be explained in the same manner, viz., by a lengthening of the

refractory phase of conduction, so that the stimulus does not reach the fibers.

And finally von Kries (22), discussing block occasioned by narrowing the connecting bridge between A and V (p. 88), does not consider that merely by this procedure can conductivity be decreased, and that in order to explain the retardation in conduction it is necessary to assume a functional change in the remaining fibers of the bridge, a view tentatively stated by Gaskell (see Gaskell, p. 66). The fact that recovery from block takes place under these conditions makes this assumption highly plausible.

From this brief summary it is clear that considering the strength of the initial impulse as always the same there are two separate and distinct possible explanations of partial block. In the first the conductivity of the fibers of the A-V connection is assumed to be impaired on account of injury (actual cutting, compression or pathological conditions), so that there is a retardation of conduction or a failure to conduct on account of the lengthening of the refractory period. In the second the cause of block is sought in changes in the V, in a decrease in irritability or a lengthening of the refractory period, so that there is a failure to contract, or a failure to respond to the impulse brought to it from the A.

Both of these explanations are of course tenable, since it must be admitted that block is sometimes brought about by pathological changes in the A-V connection and sometimes by such changes in the V. Only those cases, however, in which a modification of the connection has taken place are to be considered here. It seems impossible to get away from the assumption that in such cases the resultant block is due to the injury which the fibers still present have suffered, and that some functional change takes place which retards the impulse or blocks it. Let us consider first the type of block in which the V contracts after every second, third, etc., A systole. The length of the A-V interval is increased. The strength of the impulse reaching the V is probably decreased by the cut, as Erlanger claims to be the case when the bundle is compressed, and the V therefore fails to be excited by every A impulse because the irritability of the V does not recover after every contraction rapidly enough for it to be excited. But how is the strength of the impulse decreased if not by the injury to the remaining fibers? And how is the increase in length of the A–V interval to be explained? According to Engelmann (14 and 23) we might say that the A-V interval increases because of the decreased strength of stimulus, or that

the latent period of the V increases for the same reason. If the block remains stationary or even becomes greater this explanation might be sufficient. But when the degree of block becomes less or disappears, in other words when a stronger stimulus succeeds in getting through the connection, it is necessary to admit that the fibers of this connection have recovered in order to make this possible. Take a case of 4 : 1 block as it improves. When the reduction in the degree of block takes place directly to a 2 : 1, or to a 3 : 1 and finally to a 2 : 1 rhythm we note that the length of the A-V interval does not change or if it does it decreases slightly (see table 3). But when the block disappears and the A and V beat 1 : 1, it is almost invariably the case that the A-V interval is markedly increased at the time the transition is made. This increase in length of the A-V interval may quickly disappear or it may decrease to a greater or less extent but it usually remains considerably greater than when the beat was 2 : 1 (see table 3). The explanation of the improvement in A-V rhythm is that the conductivity of the connection fibers suddenly improves by recovery of the fibers from injury so that a stronger impulse gets through more often, or every time. Why the A-V interval is lengthened when the rhythm becomes 1 : 1 is possible of two explanations. A lengthening of the refractory period of the V so that it takes longer to contract or to be excited, or an increase in the refractory period of the fibers of the A-V connection. The second of these explanations is to me preferable. The impulse now getting through is presumably still weaker than it would have been if the cuts had not been made, and the fibers of the remaining bridge are still somewhat under the influence of injury. They cannot, therefore, conduct the impulse every time so rapidly, as they can every other time, the pause giving them an opportunity to recuperate their conductivity. The occurrence in many cases of a slight ventricular pulsus alternans just after the beat has become 1 : 1 is of interest in this connection. This again might be interpreted as evidence simply of a partial asystole, some of the fibers of the V not having returned to their full irritability, thus resulting in alternate V systoles being not so vigorous. But in many cases it is found that the A-V interval preceding the lower V systole is slightly shorter. This indicates that the A impulse gets through more quickly, and falls into the V at a time when some, or all of its fibers, have not regained their full irritability, and the following V systole is therefore lower. Next time the impulse, owing to fatigue of the connection fibers, takes longer to get through to the V. Its fibers are, therefore, more irritable

and the following systole is larger. (No claim is made that all cases of pulsus alternans are to be explained in this way.) This condition of pulsus alternans continues until, owing to the increased improvement in the connection fibers, every impulse is able to get through at maximum speed and at maximum strength.

Now as to the other type of block in which the A-V interval gradually increases until a V systole drops out, after which the same thing is repeated. Here again the strength of impulse reaching the V is decreased, and the length of the A-V interval is increased in that the impulse travels more slowly due to the injury suffered by the fibers of the remaining connection. The general increase in length of the A-V interval from cycle to cycle is due to the gradually increased refractoriness of the connection fibers. Finally a V systole is dropped. This must be considered as due to the fact that the impulse does not get through and not that the impulse getting through is sub-minimal to the V, or that it falls into, or too near to, the lengthened refractory period of the V. After the pause the connection fibers recover their irritability and conductivity, and the first impulse gets through more quickly, the A-V interval is shorter. The V systole is of course more vigorous owing to the pause. The second impulse comes to the fibers of the bridge and finds them in a state of lowered excitability and conductivity owing to the passage of the previous impulse and this second impulse is weakened in turn and slowed, and the A-V interval is therefore still longer, and so on until the impulse is completely blocked and fails to get through.

In some cases the last V systole before a beat is missed is more vigorous than the preceding ones (see (1) fig. 49, p. 187). If the dropping out were due to the A impulse falling nearer and nearer into the lengthened refractory period of the V this could not possibly be the case; but if it is due to a slowing up and a weakening in the A-V connection, then the V would have a chance, waiting for the impulse to reach it, to recover in greater measure its irritability, so that the systole following is more vigorous. The failure of the next V systole to materialize is then plainly due to the failure of the impulse to get through to the V.

Quite often this type of block may be observed to be intermediate between a 2 : 1 rhythm and a normal rhythm, and either regressively or progressively. It has already been pointed out ((1), p. 195) that further cutting of the funnel converted a case of 3 : 2 block into a 2 : 1 block, and two cases are given above (see table 3, No. 4 and 14, and

fig. 9) of the recovery of a 2 : 1 block, passing through a transitory stage of gradually increasing length of A-V interval to end with a dropping out of a V systole after which the beat becomes 1 : 1. Here again owing to recovery from injury on the part of the connection fibers the impulse able to get through the block suddenly became stronger but the connecting fibers for the time were not yet in a condition to take over every impulse without showing fatigue which they evidenced in a slowing up of the impulse and weakening it until it did not get through at all, thus causing a V systole to be missed after which a sufficient recuperation of the connection fibers took place so that the beat became normal. See also No. 18 (table 3, and figs. 8 and 1).

It has not been overlooked that a decrease in strength of stimulus alone may possibly explain both types of partial block, by the thereby relatively increased refractory state of the V, and that in the second type the gradually increased A-V interval may be explained by the gradually increasing length of refractory period of the V from cycle to cycle until an A impulse falls into it and the V cannot respond. Furthermore that the recovery from block may be simply due to an increase in the strength of the impulse getting through and the increased A-V interval to the increased length of the refractory state of the V on account of its being more often stimulated. This explanation may be the correct one, but at the present time the writer cannot rid himself of the conviction that injury to the A-V connection fibers is the ultimate cause of both of these types of block and that the failure of the V to contract is due to the failure of the impulse to reach it.

A word may be said regarding certain of my former experiments (1). In nearly all cases block was obtained by a method of gradual cutting which extended over a considerable period of time. This gave the irritability of the V time to become lowered and therefore warranted the assertion (p. 189) that the type of block characterized by a gradually increasing length of A-V interval was particularly often seen in dying hearts. Also those cases mentioned on p. 192 in which, although the A-V interval gradually increased, the V systoles did not fall out when the maximum length of A-V interval was attained, but followed the A systole after the minimal A-V interval, which gradually increased again and so on. All of these cases have been looked up and in all of them it has been found that they were truly dying, in that conditions were becoming worse instead of better, and therefore that the irritability of the V must have been decreasing. The fact noted on p. 194 that

the V began to beat regularly with a length of A-V interval equal to that at which it usually fell out simply points to the recovery of the connection fibers and also to the view above expressed that the falling out of the V is not due to the A impulse coming to it when in its refractory period.

SUMMARY

1. There is a functional differentiation in the A-V funnel of Malaco-clemmys as was found in Clemmys and in the lizards Lacerta viridis and agilis. When the auricles are partially separated from the ventricle, a connection on the right or left, though consisting of but a thread of tissue, in contradistinction to other parts of the funnel, can conduct the contracting impulse so that A-V coördination is preserved or restored.

2. Cutting the right or left parts of the funnel, however, does not necessarily produce block as was found in the other animals just mentioned.

3. Block produced by the method of cutting the A-V connection can be observed in all its transitional stages to complete recovery.

4. The cause of this block is believed to lie in the injury to the remaining fibers of the connection. This increases their refractoriness to receive and conduct stimuli. The strength of stimulus is decreased and its rate of conduction increased, until the impulse is completely blocked. The V fails to contract not because the impulse is too weak to excite it, but because the impulse does not reach it.

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SOME FEATURES OF OSTEOGENESIS IN THE LIGHT OF VITAL STAINING

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Since the time of Kölliker it has been customary to ascribe the erosion of calcified cartilage and provisional bone during endochondral ossification to the activity of a certain multinucleate cell known as the osteoclast. Although the exact manner in which this cell carried on its function, whether chemical, physical or mechanical, was obscure, it seemed possible—even probable—that the cell might be regarded as a phagocyte.

Now Goldman (1) has called attention to the reaction of the pathological giant-cell of experimental tuberculosis to certain dyes belonging to the benzidine group of colors; and has pointed out the intense staining of these cells following the introduction of dye solutions into the circulation of the infected animal. The work of Evans and Schulemann (2) showed that the staining phenomena which follow the exhibition of these benzidine dyes are closely related to—indeed are probably due to—the exercise, by certain specialized cells, of a phagocytic potentiality. These phagocytes, although anatomically heterogeneous and scattered about the body, are united by a common physiological activity in a great group. To designate them Evans borrows the term "macrophages" (Metchnikoff), and it is now recognized that their behavior toward trypan-blue and other dyes of the same class is merely the expression of a "scavenger" function which they hold in common, and which causes them to react to the ultramicrons of the dye-solution in the same way that they do to other débris which they ingest. Aside from all disputed questions concerning the histogenesis of the pathological giant-cells, the avidity with which they eat the vital dye-granules places them in the category of macrophages, and demonstrates the phagocytosis of which they are capable. If any further evidence were needed to establish their right to a place in this tissue-group the presence in their cytoplasm of bacilli or foreign bodies, which cause the

lesion of which they are a part, is sufficient to ensure their classification as able and greedy phagocytes.

The anatomical similarity between these pathological elements and the normal osteoclasts of the bone-marrow, together with the supposition of a destructive action (possibly phagocytic) on the part of the latter cells, led us to expect, in the osteoclasts of animals whose bone and cartilage was undergoing rapid erosion, an ingestive reaction, identical with that of the macrophages, toward the high molecular dyestuffs and colloid solutions. And this reaction was looked for in spite of the fact that the cytoplasm of the osteoclasts shows none of the evidences of phagocytic activity in the form of ingested pigment or red blood cells, such as are found in the macrophagic reticulo-endothelium of the bone-marrow and the other fixed phagocytes of the body. It is perfectly possible to think of these cells as able to drink in the ultramicrons of colloidal or semi-colloidal solutions (in other words the finer material subdivisions), while, because of the coarser physical state of the substance, or for other reasons, they are not able to ingest the masses of large size which, enclosed in the cytoplasm of a cell, are the usually accepted evidence of its phagocytic powers. The idea therefore presented itself of staining young and growing animals with azo dyestuffs and metallic colloids and studying the young osseous tissue; a report of such a histological study is presented in this paper.

The method used was to inject into the peritoneal cavity of young and healthy animals (rabbits, kittens and chicks) a small quantity of a sterile aqueous solution of dye or colloid, using the technique and precautions described by us in a former paper (3). The animal was allowed to live for from one to four days and was then killed and the tissues fixed in 10 per cent neutral formalin. The fixative was injected into the blood-vessels, after which the tissues were allowed to stand in it for from twenty-four to forty-eight hours. They were then thoroughly washed, dehydrated, decalcified and embedded in celloidin or paraffin.

In our former communication (3) we have described the appearance in gross of the bones of such an animal, and have suggested their value in the study of ossification centers and the development of bone, and we shall speak of gross material only briefly here.

We shall also confine our description to the findings in animals injected with trypan-blue, an azo-dye, since the color of material stained with this dye is almost unchanged by passage through the strong acids used to decalcify the bony tissue in preparation for sectioning, and

because there is no essential variation in the distribution of different benzidine dyes introduced into the body of an experimental animal.

The stain of the growing bone is in a large measure selective, resembling somewhat the osseous staining consequent upon the ingestion of madder; and while the bony skeleton is stained a very dark blue, the liver and spleen of these young animals are but lightly colored. This latter feature is especially marked in the chick. In fully developed adults exactly the reverse is true: the osseous system shows very little visible color, while the liver and spleen are the most heavily loaded places of dye-storage in the body. It would seem that the actively growing skeleton had taken to itself a large share of dye which, in the adult, would be cared for by these other tissues.

In a way this specificity of staining is what we might logically expect to find in these young animals. In them the growth curve of the skeleton is at its maximum height and the metabolism of the component elements of the osseous system must be whipped to the utmost. A study of the phenomena of vital staining demonstrates that circulating dye is always concentrated in loci of heightened metabolism, especially if, as is the case with the growing bony skeleton, the destructive or catabolic side of the life processes is prominent in the affected tissue. Thus we see the selective staining of the uterus during pregnancy, the heavy dye-deposits in the ovary which mark the situation of ova in the process of attrition, and, under pathological conditions, the special foci of dye-storage in inflammatory areas and about the borders of experimental neoplasms.

The osseous tissue is stained very darkly, and it is noticeable that this color is much more marked in the growing than in the fully developed bone. Indeed, upon close examination it is seen that especially dense staining marks the actively growing areas of the bone, as for example the ventral ends of the ribs, or the advancing borders of the membrane-bones of the skull.

These most deeply stained areas may or may not represent the so-called primary ossification centers. In fact the primary centers are stained only if they are areas of osteoblastic activity at the time of the exhibition of the dye. This is most evident in the thin bones of the developing skull where the primary centers are almost without color and the rapidly advancing edge of the membrane-bone is heavily stained.

It seemed, then, that the avidity with which the dye was taken by the growing bone had some connection with osteogenesis; and it was with the greatest interest that the sections were examined, for it was

expected that the osteoclasts would be found loaded with blue granules. Instead of this it was surprising to find that no blue at all was to be seen in their bodies.

In other words these cells are non-trypanophilic and non-phagocytic. They can therefore not be classed with the pyrrhol cells on a physiological basis.

It may be objected that the fact that the osteoclasts do not store trypan-blue does not prove them non-phagocytic, since the ordinary neutrophil leucocytes—well known phagocytes—are not trypanophilic. These latter cells however show the commonly recognized signs of phagocytosis and are able to surround bodies whose size brings them easily into the field of microscopic vision. No such evidences of phagocytic activity are ever found in the cytoplasm of the osteoclasts, though they are present in pathological giant-cells, both of the foreign body and the granulomatous type.

If the osteoclasts have any part in the resorption of calcified cartilage and bone, that part must be played either through the pressure of their bodies against it or the production of a secretion which can dissolve or at least disintegrate the calcareous tissue with which it comes in contact. If the erosion of cartilage and bone is accompanied by phagocytosis of disintegrated tissue-fragments, that operation must be carried on by other elements in the marrow-cavity.

We are probably therefore justified in including the osteoclasts in the same category with the other giant cells of the bone-marrow, all of which are trypanophobic.

The only cells which manifested the characteristic trypanophil reaction were the ordinary reticulo-endothelial cells of the marrow reticulum. Though these were everywhere present throughout the marrow cavities of the young bone, they seemed to be most numerous in the areas where erosion of cartilage and bone was going on most actively. At the margin between cartilage and bone at the epiphysis there was a narrow zone where these macrophages were very numerous, and a distinct line of them could be made out along the edge of the cartilage to which they were apparently closely applied. In a region of the new endochondral bone, just behind the advancing osseous border, viz., in the region where the new spicules of endochondral bone were being most actively resorbed, they were more thickly grouped than in the rest of the marrow-cavity.

By recourse to a method of clearing the bones the distribution of the macrophages was made even more evident.

Bones were thoroughly dehydrated in ascending alcohols and, after two changes of absolute, were immersed in benzol. After remaining in this twenty-four to forty-eight hours they were drained quickly and put into oil of wintergreen. A striking picture is then presented by such a preparation. The color of the bone becomes much darker, almost black, and the separate centers of osteogenesis are sharply marked off from one another. In a long bone, for instance, the diaphysis appears as a blue-black shaft, the thin outer shell of periosteal bone being semi-translucent. At the ends the cartilaginous caps are seen to be almost completely unstained, and the epiphyses and apophyses, where these are present, appear as distinct nodules within their capsules of cartilage. So distinct is this demonstration of the foci of osteogenesis that they may be readily studied in preparations made by this method.

If the bones be decalcified before being cleared the picture is even more striking. The density of the blue is less marked, for the bone has lost its opacity because of the extraction of the calcium-salts, and, too, a small quantity of dye appears to be lost with the inorganic material. When such a preparation is viewed through the dissecting microscope or the binocular the distribution of the dyestuff is easily studied. In bones where the margin between cartilage and advancing bone forms a plane surface, as at the ventral extremities of the ribs, a distinct blue disk appears at this situation, and, on examining the specimen with a higher power, it is seen that the blue is within pyrrhol-cells or macrophages. They are met with throughout the area of endochondral ossification, but are specially abundant just behind the advancing tip, i.e., where new bone is being most actively broken down.

Some of the deep blue stain of the growing skeleton is due however, not to granules of color in specific cells, but to a diffuse deposit of dye in the bone itself. The greatest amount of bone so stained occurs in regions where bone-formation is going on most rapidly, at the growing ends of the bone, just beneath the periosteum on the shaft, and along the borders of spicules of endochondral bone in a narrow band just beneath the covering layer of osteoblasts. The largest and most deeply stained layer in the body occurs at the ventral costo-chondral junction—a focus of very rapid osteogenesis. The corpuscles embedded in bone so stained usually have their nuclei deeply and diffusely colored and occasionally granules of dye may be seen in their cytoplasm.

The meaning of this finding is not at all clear. Diffuse tissue-staining by solutions of benzidine dyes is usually accepted as evidence of

tissue-injury, and nuclear coloration as certain evidence of cell-death. (McCurdy and Evans (4), Evans and Crowe (5), Evans Bowman and Winternitz (6)).

This staining, however, does not seem to be due to diffusion of the dye, and being in bone which has been freshly laid down, seems rather to be regarded as the result of the activity of the osteoblasts, some of which may be occasionally seen, faintly stained. It is probable that this stained bone was laid down during the time that the circulating body-fluids were charged with dye, and the stain may be comparable to the coloration of young bone in animals which are on a madder-diet. Schuleman (7) has shown that certain, and perhaps all, of the colored granulations formed in the pyrrhol-cells after vital staining with azo-dyes are the result of aggregation of the ultra-microns of the dye-solutions in the presence of electrolytes. It may be therefore that the coloration of the new formed bone is caused by the electrolytic precipitation of the injected dye, by and with calcium-salts, which have been locally concentrated to be laid down in the process of ossification. The question is being investigated further however, and the results will be published in a later communication.

The fact that the reticulo-endothelial cells are present in large numbers where absorption of bone and cartilage is going on, and that they are most numerous where this process is most active, is an indication that they are concerned in this work. We know that they are specialized to take into their cytoplasm small foreign bodies, as red blood-cells and bacteria (Kyes (8)) and pigment (Brass (9)), etc., and it seems reasonable to assume that they deal with the fragments of cartilage and bone, which are undergoing resorption, in the same manner. Whether or not these cells themselves actually break up the material is not clear, but if they do not at least it seems probable that they pick up the chips, so to speak, which other cells have produced.

What the pyrrhol-cells do with the matter which they have ingested forms a subject for fascinating speculation. They may digest it—break down complex into simple material—to be afterwards passed into the blood-stream and again utilized, or they may act as hod-carriers as well, transporting the bricks of calcium-compounds to regions where they can be built into new spicules of bone. Or they may simply prepare worthless materials so that they may be easily excreted.

If the pyrrhol-cells take up the material which has been formed from resorption of cartilage and bone, and there seems to be little doubt that they do, this suggests that such material occurs in a very

finely dispersed form, since the macrophages are so specialized as to deal most easily with physical systems of this character.

CONCLUSIONS

1. The pyrrhol-cells occur in regions where bone is developing, and are most numerous in areas where the resorption of bone and cartilage is most active. It may therefore be considered that they take some part in this process, and it is believed that they ingest the débris and assist in clearing it away.

2. The osteoclast is not trypanophilic and hence must be excluded from the class of pyrrhol-cells and their derivatives such as the foreign-body giant cell.

3. Any office of the osteoclasts in the resorption of calcified cartilage or bone must be performed by the agency of chemical solution or mechanical erosion through pressure exerted by them upon the material which is being broken down.

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THE STAINING OF AMPHIBIAN LARVAE WITH BENZIDINE DYES WITH ESPECIAL REFERENCE TO THE BEHAVIOR OF THE LYMPHATIC ENDOTHELIUM

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A new point of view has developed during the last decade concerning the inter-relationship of certain cells of the blood and connective tissue; we have had no satisfactory classification for such cells heretofore. A number of morphologically heterogeneous endothelial and connective tissue elements have been grouped together into one class by the possession of a common physiological function—that is, the ability to ingest, aggregate, and store in their cytoplasm the submicroscopic particles afloat in colloidal sols and solutions of the acid azo (benzidine) dye-stuffs. To cells having this power Goldmann (1) has given the name pyrrhol-cell. Evans and Schulemann (2) have shown that the storage of the high molecular dyes by these cells is merely a manifestation of an ability to ingest and store foreign material, existing in the form of fine physical subdivision.

The pyrrhol-cells of the adult organism are of three general types. The first type, those which are truly endothelial in nature, line the capillaries of the liver, the venules and sinuses of the spleen, the haemal-lymph nodes, and the true lymphatic glands. The second type, which are more or less fixed cells, includes those widely distributed elements designated variously as adventitia, resting wandering cells, or clasmatocytes; and those colonies of cells closely akin to them, which are found in the omentum in which situation they are referred to as taches laiteuses. To this group belong also those interesting elements designated as reticulum-cells, which occur in the pulp of bone-marrow, spleen and lymph-glands. Thirdly, we find the free macrophages, which consist of the host of round, mono-nuclear cells inhabiting the serous cavities, the mono-nuclear cells occurring so abundantly at times within the lymph-sinuses of the lymphatic nodes and occasionally in the spleen and liver; and which under experimental conditions are

found in large numbers in the latter places and even in the peripheral circulation.

The histogenesis of these various elements has become, with our increasing knowledge of their importance in the adult economy, a fascinating and tempting problem for anatomists. With the discovery of the special affinity of these cells for the diazo-dyes, it seemed as though we were on the threshold of solving the mysteries of the origin of blood and endothelium, and their relation to mesenchyme and the adult connective tissue types.

Technical difficulties soon arose however, and our dream still remained unrealized. The placenta was shown to be impermeable to vital dyes, and the direct injection of dye into the embryo to be impracticable. Introduction of the dyes into avian embryos was fraught with even greater difficulty, and repeated attempts have ended in failure. There remained no alternative but to try to introduce the dye by some means into the embryos of one of the cold-blooded vertebrates.

The object in attempting to stain amphibian larvae would be: to determine the fate of benzidine dyes when introduced into the body of the embryo, to discover which elements possess the power to phagocytise high-molecular particulate matter and to ascertain the distribution of these elements; and furthermore to show what relation these embryonic phagocytes might bear towards the pyrrhol-cells of the adult. Staining these embryos might assist us to a knowledge of the genetic interrelationship of the sessile pyrrhol-cells, and the wandering phagocytes of the mesenchyme and the serous cavities, or to shed light upon the embryology of types of cells (Kupffer cells, taches laiteuses) concerning which we know practically nothing.

Amphibian larvae, immediately after hatching, seemed suitable subjects for such an investigation although feeding experiments on the adult animal have led us to expect very little from absorption of the dyes through the alimentary tract, and the delicate organisms seemed unfit for such drastic measures as injection of the vital stain into the peritoneal cavity. So the most reasonable method appeared to be, to introduce the dye-stuffs into the water in which the larvae swam, and trust to its being absorbed through the respiratory tract or through the epidermis, which in amphibia is known to be very permeable.

For this purpose, eggs, of the common species of frogs and salamanders in the vicinity of Baltimore, were collected in April, 1916, and brought to the laboratory where they were cared for in balanced aquaria.

The species used were Rana temporaria, Hyla pickeringii and Amblystoma. The main experiments were carried out with Rana temporaria, repeated trial having demonstrated a greater immunity to the toxic action of trypan-blue, the benzidine dye used in the experiments, for this than the other two species. The author wishes to emphasize here, that trypan-blue is not the physiologically inert, non-toxic substance some writers have claimed, but that even comparatively weak solutions are definitely toxic, as will be clearly brought out by the following experiments. I have also found the dye to be definitely toxic to fish and adult amphibia when given intraperitoneally in 1 per cent solution with perfect technique.

Immediately after hatching, the larvae were transferred from the aquaria to finger-bowls containing graded strengths of freshly prepared trypan-blue solutions, made up with ordinary tap-water. In the initial experiment the strongest solution was 1 : 50. The others in decreasing strengths were: 1 : 100; 1 : 200; 1 : 400; 1: 800; 1 : 1600; 1 : 3200. The larvae were observed twice daily, morning and evening, for a few moments, as a routine, on a glass-slide in a small amount of water. The animals in the 1 : 50, 1 : 100 and 1 : 200 solutions were all dead and partly macerated the morning following the initiation of the experiment. Nearly all the larvae in the 1 : 400 solution were dead and the remainder died within the next twenty-four hours. After repeating the experiment with these strengths, the use of a solution more concentrated than a 1 : 600 was abandoned. In the 1 : 800, 1 : 1600 and 1 : 3200 solutions the animals remained alive indefinitely from two to four weeks and seemed to be little affected by the toxic action of the dye.

On the fourth day of immersion of the animals in the 1 : 800 and 1 : 1600 solutions, faintly discernible traces of trypan-blue were to be seen, in certain cells in the tail. On the fifth and the sixth days it became apparent that the trypanophilic cells, which were gradually becoming stained, lay either immediately adjacent to the course of the lymphatics, or were the cells of the lymphatic endothelium themselves. Observation of the living tadpoles on the seventh and eighth days, in a chamber such as described by Clark (3), left no doubt, but that the trypan-blue granules were within the cytoplasm of the endothelial cells of the lymphatic capillaries. Under the low power of the microscope the lymphatic system of the tail, including the caudal trunks, was brilliantly outlined in blue.

Under the high power of the microscope, in a Clark chamber, the

nuclear areas of the lymphatic endothelial cells were visible at irregular intervals along the capillary wall. These areas appeared relatively free from trypan-blue, the dye corresponding in its distribution in the cell with the granular zone of the cytoplasm; and extending in both directions, it gradually faded away into the delicate strand of protoplasm outlining the wall. The vital dye was of uniform intensity, and the granules equally numerous, no matter whether the cell was situated near the sprouting tip of a growing vessel, or lining an established channel.

After careful observation it became evident that the lymphatic endothelium had been stained quite specifically. The endothelium of the blood-vessels, many of which were distinguishable because they contained rapidly circulating blood-corpuscles, showed not the slightest particle of vital stain. Careful examination, of both mesenchyme and wandering-cells, for the slightest trace of the blue dye, was in vain; the specificity of lymphatic endothelium for the dye was complete.

It is evident, that, in the specific affinity of lymphatic endothelium in amphibian larvae, we have a means at our disposal for settling conclusively the long controversy, concerning the growth of lymphatics, in which studies on the lymphatics of amphibian larvae have played such a prominent röle in the past.

It may not be out of place here to discuss briefly the conflicting ideas about the mechanism of the growth of lymphatic vessels, which have been advanced and are now championed, by the modern students of angiology. Three views have assumed the most important places among the numbers of theories which have been evolved to explain the development of new lymphatic vessels.

1. That lymphatics may result from a transformation of blood-vessels, and that extension of the lymphatic system results from the coupling on or addition of these changed blood-channels.

2. That the endothelial cells of the lymphatics may arise from cells of the extra-vascular mesenchyme.

3. That after the initial outgrowth of lymphatics from the veins, in the form of lymph-hearts has occurred, neither mesenchyme cell nor blood-vessel endothelium contributes any further to their growth; but that they are an independent tissue, growing by division of primary lymphatic endothelium, and invading the embryonic body, as a vine climbs a trellis.

These three views, as they apply to the tadpole, can be subjected to a crucial test in the light of the discovery that trypan-blue is an

elective stain for amphibian embryonic lymphatic endothelium. Experiments necessary to this end are being prosecuted in this laboratory. Even at the present time, while these experiments with vital dyes are only in their initial stages, it is possible by their help to draw certain fundamental conclusions, which may aid in directing the study of lymphatic growth.

For example, the results of the application of this method show that the Mayer-Lewis anlagen are not modified blood-capillaries but true lymphatics, since they store vital azo-dyes in their endothelium. This is in accord with the knowledge gained from the injection and reconstruction methods, which make it seem probable that the Mayer-Lewis anlagen are parts of a continuous lymphatic vessel.

The view maintained by a second group of writers, namely, that lymphatics grow by the conversion of extraintimal and perineural spaces into lymphatic channels, by the transformation of mesenchyme into endothelium, clearly becomes untenable, since mesenchyme and lymphatic endothelial cells are shown by their reaction toward the dye to be biologically different; and conversion of one into the other becomes extremely improbable.

The observations made so far upon vitally stained larvae, substantiate and add additional proof to the interesting observations by E. R. Clark (4) on the growth of lymphatics in the tadpole's tail. The author is able to confirm Clark's view that these lymphatics grow only by sprouting, and by sending out fine protoplasmic processes, which gradually become definite lumen-containing sprouts. Furthermore the author also agrees with Clark in maintaining that blood-vessels, and mesenchyme and wandering-cells, never contribute to the formation of lymphatic endothelium in this locality; but that each of these tissues has an independent, characteristic existence.

The staining of the larvae became so intense by the eighth day that even to the unaided eye a blue color was evident in the tail. A diffuse blue stain of the peritoneal cavity became apparent about the third day; but it seemed plausible to attribute this to the presence of dye in the intestinal contents, especially as a plug of faecal material stained deep blue could always be seen in the anal gut.

The animals were fixed at successive intervals from the third until the tenth day in 10 per cent neutral formalin. The material was dehydrated, imbedded in paraffin, cut, and stained with Mayer's carmalum.

Study of the sections seems to show conclusively that the epidermis does not serve as a portal of entry for the dye, since there are no visible

traces of trypan-blue within or between the cells of this tissue. It seems more probable that the stain gains admission to the body through the alimentary canal, because in certain regions of the gut, the entire intestinal mucosa and submucosa are colored a deep diffuse blue and occasionally definite granules of dye are distinguishable in these layers. The embryonic intestine behaves radically differently towards the dye than does that of the adult, which under no circumstance, has been known to absorb the dye or to have visible particles of stain in the cells of the mucosa. Large amounts of the blue are aggregated in the cytoplasm of the vacuolated, mucoid epithelial cells, which line certain regions of the gill-pockets (notably those adjacent to the respiratory mucosa) and it is interesting to note the rich supply of lymphatics in this region, all of whose endothelial cells are loaded with trypan-blue. The respiratory epithelium appears to be practically free from trypan-blue granules, although in some instances areas may be found in which these cells have become actively phagocytic towards the stain.

As yet it is impossible to state positively whether the staining of the intestine represents an attempt on the part of the organ to absorb the dye, or whether this coloration is evidence of an excretion process or whether, as seems most probable, the absorption and excretion of the dispersed dye are not proceeding simultaneously through the intestinal epithelium. However, the mucous nature of the epithelium of the gill-pouches makes it seem probable that in this region we may be dealing with the excretory phase of vital staining.

A distinct attempt on the part of the body to excrete the dye, is evidenced by the remarkable appearance of the kidneys. The epithelial cells of many of the tubules are uncolored or contain only traces of the dye, whereas in other tubules the cells seem loaded to their full capacity. It is possible to find normal cells in all stages of transition between the two conditions. In still others there are degenerative changes, where judging from the accumulated stain, the cells are apparently taxed by the tremendous influx of foreign material, beyond their physiological limit. The epithelium in this instance is swollen to three or four times its normal height, the lumen of the tubule nearly obliterated, and the cytoplasm filled with clumps of dye and numerous vacuoles of varying sizes. It is not improbable to suppose, that the toxic action of stronger solutions of diazo-dyes upon poikilothermal vertebrates, can be accounted for by an identical, but more extensive degeneration of renal parenchyma, following an overtaxing of the kidney.

It is clear from microscopic study of the sections that the dye has

been stored by three types of tissue as follows: by the lymphatic endothelium in its entirety, the Kupffer cells lining the sinuses of the liver, and by groups of large, round, mono-nuclear pyrrhol-cells occurring in the mesentery and omentum, not unlikely homologues of the taches laiteuses of higher vertebrates.

There is no phagocytic activity shown toward the dye by any type of cell elsewhere in the organism. There are no trypanophylic clasmatocytes in the connective tissue, nor are there any free "macrophages" discernible, either in the serous cavities, or in the blood-stream. There is no vital stain in the spleen or blood-forming tissue, and the circulating blood-elements do not contain a trace of the dye.

The lymphatic system throughout the organism is conspicuous by the brilliant trypan-blue granules displayed in the cytoplasm of its endothelium. Here, as in the living specimens, there is no difficulty in distinguishing lymphatics from the blood-vessels because of the absence of dye in the endothelium of the latter.

The nuclei of the lymphatic endothelium are small, flattened, lense-shaped, or somewhat irregular, and stain very deeply with carmalum; they never contain dye-granules, while the cytoplasm is conspicuous by the quantity of vital stain which it exhibits. The dye however appears to be in larger clumps and masses than in the living cell, where it is distributed in much finer and more diffuse particles. Besides the trypan-blue the cells contain fine pigment-granules, which are also visible in vivo, and which would appear to be normal for this species. The vital stain is limited in the stained section, as in the living cell, to the granular zone surrounding the nucleus, and does not extend far into the finer protoplasmic filaments of the sprouting tip of the lymphatic vessel, or into the strands of protoplasm extending from nucleus to nucleus and forming the capillary-wall. One receives the impression here, as well as in the living, that the dye granules have a tendency to remain near the more physically inert nucleus, rather than to enter into the plastic and markedly amoeboid pseudopods of the cell.

The liver is characterized in all the sections studied by the large quantity of dye which it contains. Here it is the endothelium of the vascular sinuses, the Kupffer cells, which house the dye. In the amphibian larvae the number of these cells relative to liver-parenchyma would appear to be very great. The liver cells themselves contain appreciable amounts of dye, a phenomenon seen in other animals at times; and possibly attributable to the fact that these cells absorb some of the dye in the presence of an excess in the blood-stream, which

cannot be cared for by the cells, whose chief function it is to phagocytise particulate matter.

The third place of storage of the dye-granules, in the larval amphibians, is in the colonies of pyrrhol-cells in the mesentery and omentum. Some of these cell-aggregations seem to be true homologues of the taches laiteuses of higher vertebrates, others are merely accumulations of primitive wandering-cells peculiar to amphibian larvae in this locality. These cells are round and relatively large, having a single, round, eccentrically placed nucleus. Under no circumstance does the nucleus store the dye, but the cytoplasm, besides a quantity of brownish-yellow pigment-granules, displays an enormous amount of dye in clumps of differing dimensions and intensity.

The above results apply to all of the species studied, although the results with Hyla pickeringii were not nearly as satisfactory as with Rana temporaria, owing to the fact, explained above, that the toxicity of the dye towards the latter was very much greater, the animals succumbing before a brilliant vital stain was obtained. Amblystoma, although surviving longer than the other species in 1 : 1000 and 1 : 1200 solutions of the dye, had several drawbacks, which militated against their use. For example, the study of the lymphatics in vivo was impracticable, although their endothelium has the same affinity for trypan-blue, because of the dense, opaque chromatophores and melanophores which characterize this species of amphibian, and also on account of the slow rate of absorption of the dye, the endothelium frequently showing no trace of dye before the twelfth or fourteenth day.

The question now arises as to the significance of this rather striking distribution of trypan-blue in amphibian larvae. Leaving out the intestinal tract, which is concerned only in the transmission of the dye to its final destination, and also the kidney, where there is merely an attempt on the part of the body to rid itself of an excess of foreign material, the only cells found storing the dye are the lymphatic endothelium (throughout its entire extent), the specialized endothelium of the liver capillaries, and colonies of pyrrhol-cells in the mesentery and omentum. May we regard the lymphatic endothelium of the amphibian larva as an intermediate stage in the phylogeny of the pyrrhol-cell? Could we but vitally stain the amphibian embryo at a still earlier period, it would not be startling to find the blood-vascular endothelium similarly stained. This is borne out by the fact that, after experimental injury to the blood-vessel wall, its endothelial lining-cells apparently resume a more primitive and embryonic function, as evidenced by the absorption of the vital-

dye by the damaged endothelium. It would seem plausible, then, to infer that this phagocytic potential may be common to all endothelium in very early embryonic life, and that it may be lost progressively by the large majority of endothelial cells during their later development, and finally retained only by scattered groups of endothelial cells in the liver, bone-marrow, spleen and lymph-glands. What relation the fixed phagocytes in adult connective tissue bear to this primitive phagocytic endothelium is not at this time clear; but it is to be hoped, that the additional work now under way, on amphibian and other embryos, may eventually settle this much discussed question.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

II. The Influence of the Vagus Nerves on the Fractionate Contraction of the Right Auricle

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I. INTRODUCTION

It is generally conceded that stimulation of the vagus nerves causes a marked reduction in the amplitude of auricular contraction in mammals. Experiments similar to those reported by MacWilliam in 1888 (1) have been frequently reduplicated by experimental investigators. When the movement of a point on the auricular surface (usually the appendage) is transmitted to a recording lever or a tambour transmission system, the amplitude of the recorded curves becomes gradually smaller during weak vagal excitation until, in some instances, complete stoppage occurs. Upon cessation of stimulation the reverse process follows; the beats gradually increase in amplitude until a range equal to or exceeding the normal occurs.

In the foregoing paper (2) it was pointed out that not only does the "suspension curve" not represent a true record of auricular shortening, but the myogram recorded by an efficient myocardiograph from two approximating points is not necessarily an index of the contraction process as it affects the individual units of auricular muscle. Evidence was presented which favored the conception that the contraction process spreads over the auricle in the wake of the excitation wave in such a manner that the portions nearer the sinus node begin to relax before the more distal portions have ceased to contract. The interval that any unit of cardiac tissue remains in the contracted state was designated as the *fractionate contraction*. When the approximation of two distant points is recorded, the shortening, designated as the *mechanical contraction*, represents the algebraic sum of fractionate contractions and relaxations between these points.

It is evident that the influence of the vagus nerves on the contractile function of the auricular tissue can be satisfactorily tested only by utilizing the *fractionate contraction*. While it is impossible to obtain a record of the contracted state of a small imaginary unit of auricular muscle, it seemed probable that such a record of the fractionate contraction could be approximately realized by attaching the arms of the miniature myocardiograph to points only 3 or 4 mm. distant on the auricle. Such proved to be the case.

In figure 1 are shown two exactly superimposed records of the mechanical contraction (lower curve) recorded from points 18 mm. apart on the anterior surface of the right auricular and the fractionate contraction (upper curve) recorded for two points 2.5 to 3 mm. apart near the distal arm of the other myocardiograph attachment. The two curves show distinct differences in contour. The myogram of the fractionate contraction differs from that of the mechanical contraction in the following respects:

1. The onset is delayed about 0.02 second (e.g., point A).
2. The gradient of the descending limb is straight.
3. The apex is acute.
4. The ascending limb (relaxation) coincides with that of the mechanical contraction.

II. EXPERIMENTAL RESULTS

The right and left vagus nerves were stimulated in a total of 26 experiments. The strength of current was so adjusted that a moderate retardation of the auricle was induced.

Vagus excitation increases the amplitude of the first vagal beat but progressively decreases the amplitude of succeeding beats. This statement applies alike to the mechanical and fractionate contractions, as illustrated in figure 1. The increase in amplitude of the first vagal beat, which appears not to have been noted by less sensitive methods, was present without exception when a moderate rate of retardation occurred. This increase is striking when a long diastolic interval precedes the first vagal beat but requires measurement to discover when the cycles lengthen very gradually. It is entirely absent, as shown in figure 2, when the rhythm during vagus excitation remains unaltered. This shows that a considerable latent period elapses before the depressing effect of vagus excitation becomes predominant over some other influence tending to augment the contraction. What this influence is—whether due to the beneficial influence of the longer

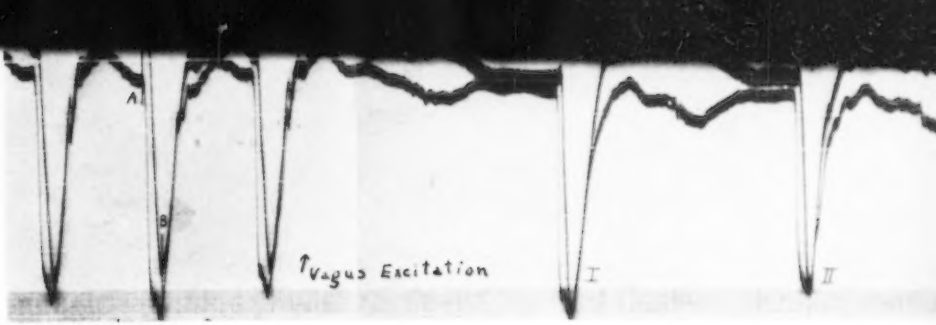


Fig. 1. The fractionate contraction (uppermost record) and mechanical contraction (lower curve). Letters referred to in text.

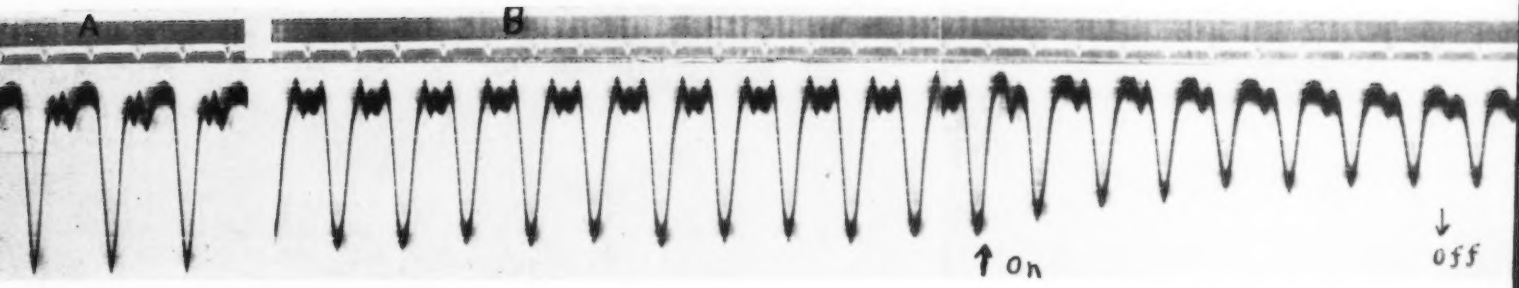


Fig. 2. *A*, Record of normal fractionate contraction of right auricle. *B*, The same. Rhythm determined by series of rhythmic artificial

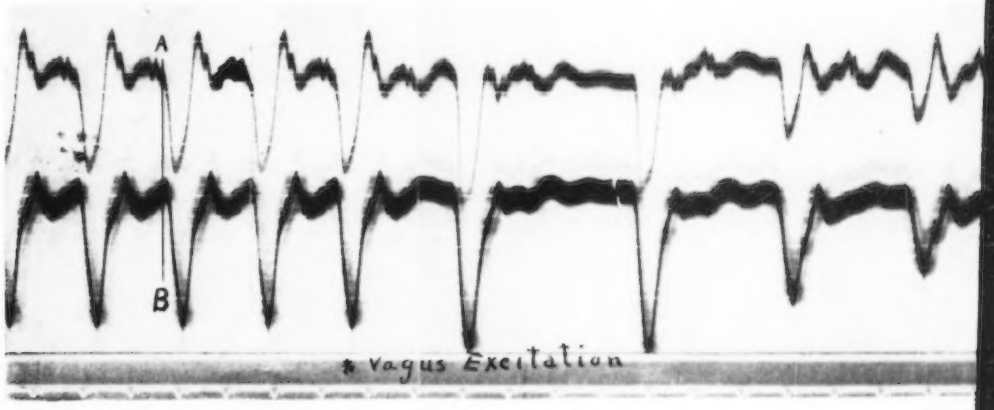


Fig. 3. Two records of fractionate contraction of right auricle. Upper record from proximal region; lo right auricle. Before and during right vagus excitation.

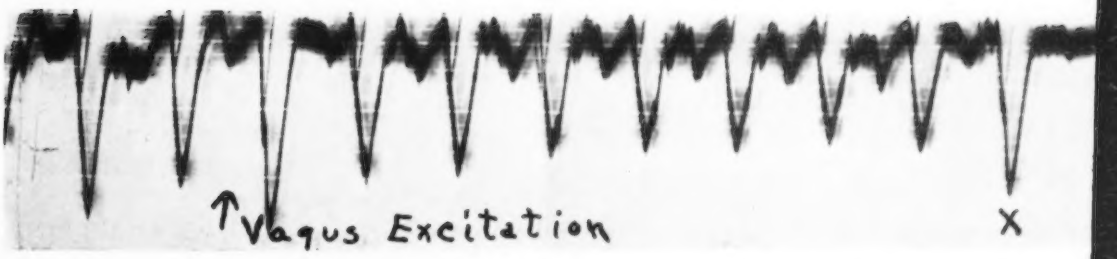
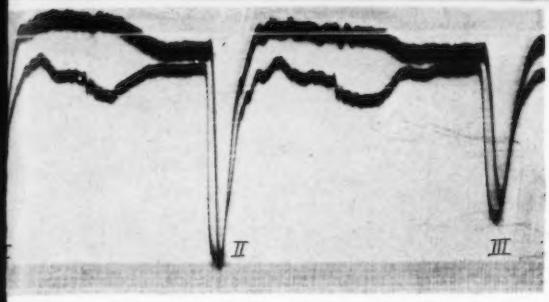
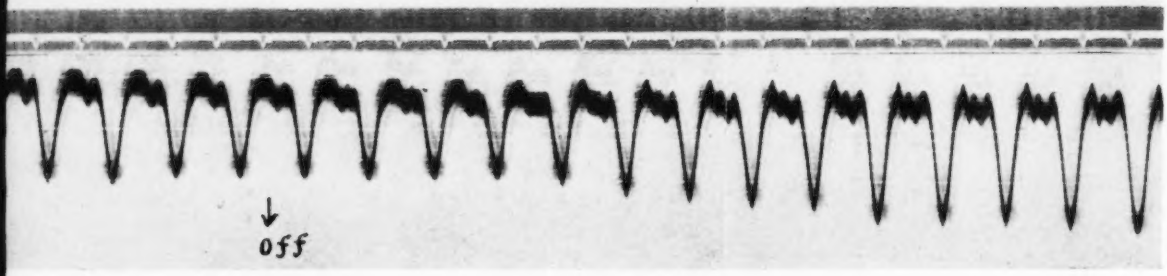


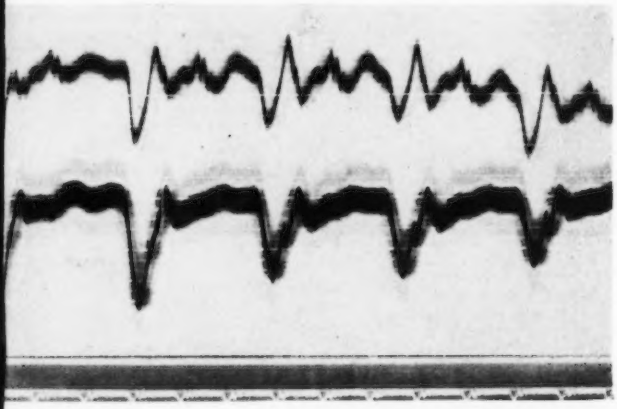
Fig. 4. Record of the fractionate contraction of the auricle; rhythm artificially maintained by rhythmic, minimal Amplitude first decreased without affecting rate; later (beat x) artificial stimuli became subminimal



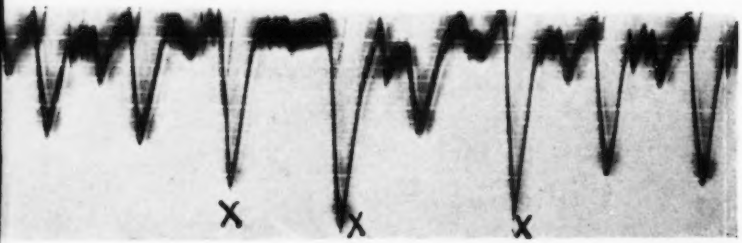
echanical contraction (lower curve) of the right auricle.
to in text.



ed by series of rhythmic artificial stimuli. Before, during and after right vagus stimulation.



record from proximal region; lower record from appendage of
g right vagus excitation.



aintained by rhythmic, minimal, external stimuli. During vagus excitation.
ficial stimuli became submininal and slower rhythm occurred.

rest period which precedes, or to the simultaneous stimulation of antagonistic nerve fibers in the vagus—can not be absolutely determined from these experiments. The fact that it is dependent upon the lengthening of a preceding diastole strongly favors the former view.

Figure 1 shows that after the first vagal beat the amplitudes of the mechanical, as well as of the fractionate contractions, progressively decrease. Hence, it may be assumed that the decrease in the amplitude of the mechanical contraction is due to a corresponding decrease in the contraction of each fractionate part of the auricular muscle.

The depressant effect on the contraction of the right auricle is exerted by both vagi, is independent of any dromotropic action, but is always associated with variations in rate. In figure 3 of the previous article, the effect which stimulation of the left vagus produces on the mechanical contraction is shown. Auriculo-ventricular block was present, showing that this nerve exerted a marked dromotropic action on the *a-v* bundle at the time that the depression of auricular contraction occurred. A comparison with figure 1 of this article, where no dromotropic action is evident, shows that the depressant action is exerted equally by the two vagi and can not be secondary to changes in conductivity. Following the lead of Pawlow (3) and others, an attempt was made by stimulating various intrathoracic branches of the right vagus nerve to obtain an effect on amplitude independent of variations in rhythm, but no such dissociation could be elicited.

The depressant effect of vagus excitation persists when the rhythm of the auricle is maintained by regularly applied electrical stimuli. The first portion of figure 2 shows a series of normal fractionate contractions of the proximal end of the right auricle. The next section shows the fractionate contraction resulting when the normal pacemaker was supplanted by an artificial series of electrical break shocks given at a rate somewhat more rapid than the normal rhythm. Owing to the shorter diastole their amplitude is smaller. While this excitation was maintained the right vagus was stimulated. The customary reduction in amplitude of the artificially induced contractions occurred, showing that the variation in amplitude occurs independently of the chromotropic action of the vagus.

The depressant vagus effect is exerted equally and simultaneously on the fractionate contractions of the proximal and distal portions of the right auricle. Figure 3 shows simultaneous records of the fractionate contractions taken from the proximal (upper curve) and distal portion (lower curve) of the right auricle. The lines *A B*, representing synchro-

nous points, show that contraction and relaxation of the proximal portions of the auricle precede that of the more distal portions. Vagus excitation in this instance is followed by two larger beats after which the characteristic depression in amplitude results in both curves. Recovery which, owing to the length of the record, can not be reproduced, followed in reverse fashion but synchronously in both curves.

Vagus excitation does not alter the duration of the fractionate contraction. On account of the sharp apex of the *fractionate* contraction curves there is no difficulty in establishing the precise onset and termination of auricular contractions. Careful measurement of curves, such as those shown in figures 1, 2 and 3, shows absolutely no variation in the interval of fractionate shortening during vagus stimulation. The gradient of the down stroke becomes, therefore, less steep. The mechanical contractions recorded from more widely separated points show small variations in the duration of contraction. Sometimes there is a lengthening and sometimes a shortening of the contraction periods, but as these variations do not exceed those normally occurring, they are apparently not significant. The reason for these variations can probably be found in the explanation advanced in the preceding paper—that the apex does not represent the end of all contraction processes, but a balance of contracting and relaxing fibers. As shown in figure 1, this balance shifts slightly during vagus excitation. In the normal beats previous to excitation the apex of the fractionate contraction falls beyond the apex of the mechanical contraction. In the first two vagal beats they coincide and in the third beat it falls on the ascending limb of the mechanical contraction. These results emphasize the importance of utilizing approximately fractionate contractions in studying the fundamental properties of the auricle muscle.

Vagus excitation reduces the irritability of auricular muscle but this can not account for the final reduction in amplitude. If the auricle is excited by a series of minimal break shocks applied at a rate exceeding the normal rhythm and the vagus nerve is then stimulated, these artificial stimuli may become subminimal, as shown by the fact that the auricle lapses into a rate equal to or slower than its normal. An indication of this is shown in figure 4. After vagus stimulation the amplitude decreases in the typical manner. The artificial tempo is at first unaltered, as in figure 2; later, as shown by the beats x, x the artificial stimuli become subminimal and slower beats occasionally appear. This in turn may be followed by a resumption of the normal or a slower rhythm. While such experiments show that the vagus depresses the

irritability of auricular tissue, evidence is lacking that this depression occurs as early as the reduction in amplitude. Diligent efforts to demonstrate such a synchronous and early reduction of irritability have failed. As far as experimental evidence goes, it appears that the reduction of irritability follows the reduction in amplitude and is not responsible for it.

The auricular depression following vagus stimulation is independent of changes of initial intra-auricular pressure and initial length. In figure 3 of the preceding paper of this series is shown a record in which, during vagus stimulation, a gradual dilatation of the auricle accompanies the increase of intra-auricular pressure. The initial length of each successive beat increases, as shown by the distance of the points *A* from the base line. A similar result is shown in figure 2. As the combined increase in initial length and initial tension tends to increase the amplitude of contraction, it is clear that the reduction of amplitude has occurred in spite of these factors.

This is not the common effect of vagus stimulation on the initial length, as indicated by these experiments. In 17 out of 26 cases the initial length decreased shortly after the onset of stimulation (fig. 1) and an analysis of those tracings, accompanied by an intra-auricular pressure record, shows that it occurs in spite of an increase in initial pressure within the auricle. These cases all show the same gradual reduction in the amplitude of the mechanical or fractionate contraction of the auricle.

III. THE NATURE OF THE VAGUS INFLUENCE OVER AURICULAR CONTRACTION

The vagal depression of auricular contraction has been assigned to various influences. It is the prevailing opinion that it is not secondary to changes in the intra-auricular or the coronary pressure. MacWilliam (1) found the depression entirely independent of the degree of stasis or the height of intra-auricular pressure. This was confirmed by these experiments; in fact, when an increased initial pressure and decreased initial length occur one would expect these influences to augment rather than reduce the amplitude of contraction. Langendorff (4) found that the reduced amplitude occurred in perfused hearts without variation in coronary pressure and the writer obtained similar records years ago.

The most plausible view attributes the depressing action of the vagus

nerve to a direct influence on the auricular muscle. The nature of this influence has been the subject of extensive experimentation and discussion. Does the vagus nerve depress the function of contractility directly or is this function inhibited as a secondary result of a primary alteration in the functions of rhythmicity, conductivity or irritability? The evidence in regard to the proposition that such secondary causes are concerned may be briefly reviewed:

1. That the reduction in contraction is secondary to changes in rhythm. The reduction in amplitude of the auricular contraction is usually associated with a decrease in rate. According to François Franck (5) and Bayliss and Starling (6), it is possible by vagus excitation to elicit one phenomenon without the other. This could not be confirmed in this research. When, however, the auricle was excited by electrical stimuli well above the minimal in strength, excitation of the vagus nerve produced a reduction in the amplitude without, of course, modifying the rate. These results show the complete independence of changes of rate and amplitude.

2. That reduction in contraction is secondary to changes in conductivity. As postulated by Englemann (7), a depression of conductivity may reduce the amplitude of contraction in three conceivable ways:

(A) The velocity of the excitation wave may be decreased so that the interval within which the proximal and distal units of auricular tissue enter into contraction, increases. In opposition to such an assumption Englemann cites the following evidence:

(1) No delay can be observed between the contractions of different portions of the frog's auricle.

(2) The duration of the contraction period in the frog is shorter and not longer during vagus excitation (8), (9).

Both of these observations have been contradicted, however, in the case of mammals; the former by Hering (10) who professed to *observe* that during vagus excitation the contraction wave progressed more slowly in the rabbit's auricle; the latter by Klug (11) who found that the duration of auricular contraction was longer after vagus excitation. Neither of these observations is corroborated by later work. Of more value than the observations of Hering because they were carried on by methods permitting greater accuracy, are the results of Lewis, Meakins and White (12). They found that the rate at which the impulse travels over the auricle is, to judge from the spread of the electrical wave, not modified by vagus stimulation. Klug, owing to the method employed, was not able to record the myogram of the auricle correctly. The

evidence submitted in this paper shows that the duration of the fractionate contraction is unaltered by vagus stimulation.

(B) The excitation wave may be blocked so that it fails to reach the most distal portions of the auricle. In consequence, a shorter length of auricular tissue would contract and the amplitude would be reduced. This view has been discounted

(a) By the failure to observe a quiescent region in the more distal portions of the auricle (Englemann);

(b) By the fact that a negative variation does spread to the more distal portions (Lewis, Meakins and White);

(c) By the fact that separate records of the proximal and distal portions of a partially clamped amphibian auricle may show a decrease in amplitude in the distal segment during vagus stimulation without a corresponding decrease in the proximal region;

(d) By the fact, shown in this research, that the records of the fractionate contractions taken from the distal and proximal portions of the mammalian auricle undergo a simultaneous and proportionate decrease during vagus stimulation.

(C) Without any impairment of longitudinal conduction, the cross conduction through cardiac fibers may be impaired during the vagus stimulation. In consequence, a smaller cross section area would contract. The fact that widespread extraneous stimuli applied to the auricle during vagus excitation gives rise, not to a larger, but to a smaller contraction, is cited by Englemann against this possibility.

The evidence of experimental investigation is evidently opposed to the assumption that the reduction in amplitude of auricular contractions is secondary to disturbances of conductivity.

3. That the reduction in amplitude is secondary to changes in irritability. The observations of MacWilliam, Englemann and others, that stronger stimuli are required to elicit an extra contraction during vagus stimulation, are usually cited to show that in the mammalian and amphibian auricle a distinct bathmotropic effect is induced by vagus stimulation. The difficulty in applying this method consists in exciting the auricle in exactly the same phase of relaxation. More distinct proof may be gained by exciting the auricle by a series of minimal artificial stimuli. Upon vagus excitation, as shown by Englemann in the case of the frog's heart, and extended to the mammalian heart in this research, these stimuli become subminimal, indicating a reduction of irritability of the cardiac tissue. It was pointed out, however, that no experimental evidence of a reduced irritability could be obtained

as early as the reduction in contraction amplitude. While the possibility that the decreased irritability following vagal excitation is, in part, accountable for the reduction in amplitude must be borne in mind, experimental evidence does not favor this explanation of the early reduction in amplitude.

By exclusion, we are forced to the conclusion that the vagus exerts a depressing influence on the contractility of auricular muscle. There is also direct evidence that such a negative inotropic effect exists. Englemann showed that when the frog's auricle is stimulated artificially at a rate exceeding that set by the pacemaker and variations in rhythm and irritability are thus avoided, vagal stimulation still decreases the amplitude of auricular contraction. This has also been shown to be true for the fractionate contraction of the mammalian auricle by the experiments reported above.

IV. CONCLUSIONS

1. The depression of the mechanical contraction of the mammalian auricle which follows vagal excitation is due to an actual decrease in the contraction of the individual cardiac units.

2. The decrease is not secondary to changes in rhythm or to alterations in conductivity.

3. The decrease may be favored by the depression of irritability produced by vagus stimulation but is primarily due to the depressant effect of the vagus on the function of contractility.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

III. The Time Relations of Auricular Systole

I. THE EVENTS OF AURICULAR SYSTOLE AND THEIR RELATION TO VENTRICULAR SYSTOLE

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In an earlier paper (1) the term "auricular systole" was restricted to the interval elapsing between the inception of contraction and the maximal shortening of the entire auricular musculature. Evidence was presented which showed why it is impossible to calculate this interval, either from the waves of the intra-auricular pressure curve or from the myogram taken individually. Auricular systole extends from the first rise of intra-auricular pressure to the apex of the myogram curve. Its duration averages roughly 0.1 second.

The interval elapsing between the end of auricular systole and the beginning of ventricular systole (determined from the rise of the intraventricular pressure curve) may be designated as the *intersystolic interval.*[1]

As shown in figure 1, this interval may be non-existent, the rise of the intraventricular pressure coinciding absolutely with the termination of the myogram curve. Under abnormal conditions, as shown in the fourth premature ventricular systole, the rise of intra-ventricular pressure may even precede the termination of auricular systole. As a rule, however, a short interval is found.

As shown by the experimental results in column 1 of table 1, the intersystolic period varies from 0 to 0.068 second, averaging 0.024 second. This shows that the contraction and relaxation of the mammalian chambers are so timed that very little or no time is lost between

[1] In thus defining the intersystolic interval the writer is aware that the term is employed frequently but with little reason to designate the interval elapsing between the onset of auricular and ventricular systoles; i.e., synonymously with A_s–V_s interval.

the termination of auricular systole and the inception of ventricular contraction.

The intersystolic interval added to the period of total systole gives the A_s-V_s interval; i.e., the time elapsing between the onset of auricular systole, as evidenced by the first rise of intra-auricular pressure, and the onset of ventricular systole, as evidenced by the first rise of

TABLE 1

EXPERIMENT	1 Intersystolic interval	2 Total systole	3 A_s-V_s interval	4 Ratio Intersystolic A_s-V_s interval	5 Interdynamic interval
	second	*second*	*second*		
C75 VIII	0.068	0.070	0.138	1 : 2	
C77 VI	0.020	0.126	0.146	1 : 7.3	
C78 III	0.011	0.08	0.091	1 : 8.2	
C79 IV	0.045	0.126	0.171	1 : 3.8	
C80 XV	0.063	0.077	0.14	1 : 2.2	
C84 I	0.040	0.14	0.18	1 : 4.5	0.014
C89 I	0.012	0.10	0.112	1 : 9.3	
C93	0	0.124	0.124		0.05
C96	0.010	0.102	0.112	1 : 10.2	
C97	0.028	0.14	0.168	1 : 6	
C98	0	0.108	0.108		
C100 II	0.014	0.130	0.144	1 : 10.2	
C103 XI	0.050	0.078	0.128	1 : 2.56	
C116 I	0	0.11	0.11		0.044
C124	0.002	0.09	0.092		0.025
C70 VI					0.075
C87					0.070
C88					0.013
C90					0.013
C91					0.038
C121					0.075
Average	0.024	0.106	0.13		0.042

intraventricular pressure. This period (column 3) ranges from 0.091 to 0.18 second and averages 0.130 second. Since the period of systole varies independently of the intersystolic interval, it is apparent that *the A_s-V_s interval is not an accurate index of the inter-systolic interval.* In column 4 of table 1 it is shown that the ratio between these two periods varies from 1 : 2 to 1 : 10.2.

The intra-auricular pressure continues to rise only during the early half of systole and declines during the latter portion. This was first pointed out by Ewing (2) and was confirmed by photographic tracings of the writer (1). The interval between the maximum intra-auricular pressure and the onset of ventricular systole may be designated as the *interdynamic interval* because any influence that intra-auricular pressure might have upon the initial intraventricular pressure steadily diminishes as the duration of this period increases. This interval is obviously longer than the intersystolic interval and in dynamic considerations is much more important. The results of column 5 in table 1 show that this period varies from 0.013 to 0.075 second, averaging 0.042 second. It is proportional neither to the A_s-V_s interval nor to the intersystolic period, as is evidenced by a comparison both of average figures and of the actual figures in the same case.

II. THE RELATION OF AURICULAR SYSTOLE TO THE WAVES OF THE VENOUS PULSE AND THE ELECTROCARDIOGRAM

In the experiments analyzed in this and a preceding paper a considerable variation existed, not only in the duration of various phases of auricular systole but in their relation to ventricular contraction. The possibility must always be borne in mind that this may be due to variable disturbances incident to exposure and cooling of the heart, abnormal circulatory derangements consequent upon an abolition of the negative intrathoracic pressure, etc. It would, therefore, be very desirable to obtain data in regard to the auricular systole and its relation to ventricular systole from unoperated animals and man. Inasmuch as the time relation of the venous pulse and electrocardiogram are often considered of value in such determinations, it is important to establish their relations to the dynamic changes occurring within the heart chambers.

In obtaining multiple records of different cardiac events by photographic projection, it is important, as has been many times emphasized (3), (4), (5), that the optical mirrors must be aligned in the same vertical plane if the records are to be precisely over one another at any moment. As this is sometimes difficult, proper corrections for displacements must be made by briefly exposing the paper while it is not moving. The relative point at which each record starts after opening the shutter may be used as a correction provided the photokymograph is so constructed that the paper is running evenly at the

moment when the shutter is first opened and provided also that the shutter exposes all parts of the slot equally and simultaneously.[2]

Relation to the waves of the venous pulse. The time relations of the presystolic wave of the external jugular pulse recorded from the lower cervical region by a covered receiving tambour connected with a Frank capsule, are shown in figure 2. The jugular pulse consists of a presystolic wave *P*, a systolic impact *S*, followed in this case by a large after oscillation due to a light apposition of the receiving tambour. The second sound occurs at S_2 after which, owing to the short diastole, only a small diastolic wave follows. In this case, the onset of auricular systole, as indicated by the rise of intra-auricular pressure, preceded the rise of the jugular pulse 0.06 second. Considerable variation of this figure exists, however, as shown in table 2. The actual delay is

TABLE 2

EXPERIMENT	TOTAL SYSTOLE	DYNAMIC INTERVAL	PRESYSTOLIC WAVE (RISE)	DELAY IN PRESYSTOLIC WAVE
	second	*second*	*second*	*second*
C75	0.070	0.041	0.045	0.092
C77	0.126	0.038	0.043	0.056
C78	0.080	0.042	0.043	0.038
C79	0.126	0.062	0.055	0.071
C96	0.102	0.050	0.049	0.048
C121	0.100	0.037	0.038	0.060
Average	0.101	0.045	0.0455	

probably largely determined by the distance from the auricle. When the pulsations are recorded directly from the superior vena cava the delay becomes very small.

The duration of the rise of the presystolic wave is apparently shorter than the total systole but corresponds closely to the dynamic interval. This is shown by a comparison of the rise with the total systole, *x-y*, and the dynamic interval, *a-b* (fig. 2), as well as by the figures given in table 2.

Inasmuch as the summit of the presystolic wave (although delayed) corresponds to the summit of the auricular wave of the intra-auricular pressure, and as the latter does not represent the end of auricular sys-

[2] The onset of the tracings is not indicated in the reproductions of this article owing to the fact that they were slightly shortened during the process of engraving.

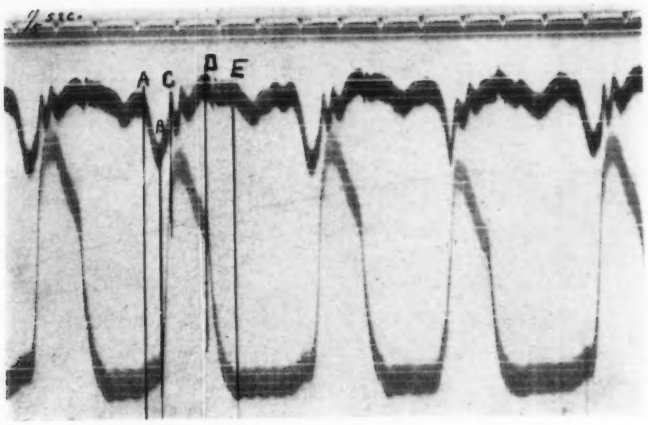

Fig. 1. Synchronous records of right auricular myogram and left intraventricular pressure. Points exactly superimposed. *B*, end of auricular systole; *C*, onset of ventricular ejection; *D*, end of auricular ejection; *E*, end of relaxation. Showing absence of an intersystolic interval and correspondence of changes between intraventricular pressure and accidental auricular movements. In the third beat a premature ventricular systole occurs before the end of auricular systole.

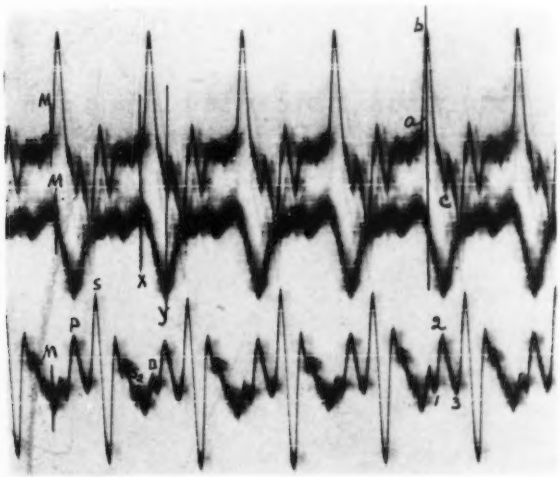

Fig. 2. Right intra-auricular pressure (upper curve); *a*, beginning of auricular systole; *a-b*, dynamic interval; *c*, systole variation. Right auricular myogram (middle curve); *x-y*, period of auricular systole. External jugular pulse (lower curve); *P*, presystolic wave; *S*, systolic wave; S_2, second sound; *D*, diastolic wave. *1*, *2*, *3*, duration of auricular wave. Lines *M* show displacement of points due to alignment of recording apparatus.

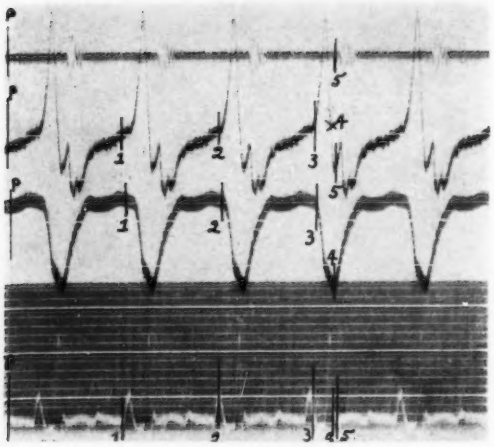

Fig. 3. Ventricular sounds (upper curve); right intra-auricular pressure (second curve); right auricular myogram (third curve); electrocardiogram, lead II (bottom curve). *P*, relative alignment of points. Numerals *1, 2, 3, 4, 5*, synchronous points on records.

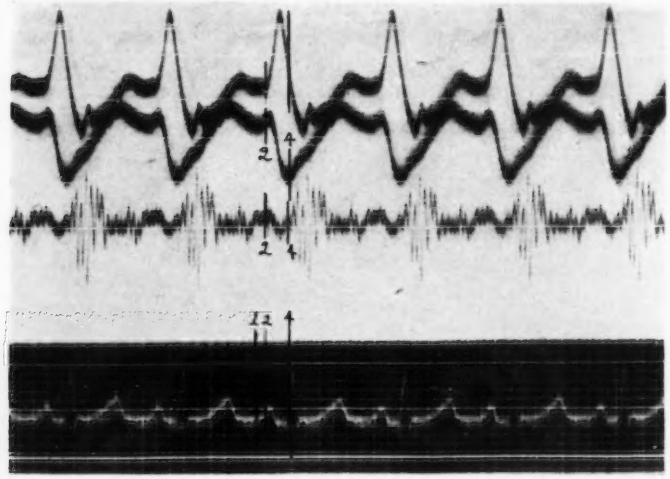

Fig. 4. Right intra-auricular pressure (upper curve). Right auricular myogram (second curve). Ventricular sounds (third curve). Electrocardiogram, lead II (bottom curve). Numerals same as in preceding figure. Time 0.05 second.

tole, *it is obviously impossible to estimate the intersystolic interval from the jugular waves.* Are we justified in using the interval extending from the summit of the presystolic wave to the arterial impact as an index of the *interdynamic interval?* To obtain equality it is necessary that the delay occasioned in venous transmission should be very nearly offset by the delay in arterial transmission plus the isometric interval. In figure 2 the interdynamic interval, *b-c*, equals 0.06 second; the equivalent period, 2-3, in the venous pulse also equals 0.06 second. Similar relations are shown in results arrayed in table 3. Evidently, the *a-c* interval averages about 0.015 second less than the true inter-dynamic interval. An approximation of the interdynamic interval may therefore be made only roughly in normal rhythms.

TABLE 3

EXPERIMENT	INTERDYNAMIC INTERVAL ESTIMATED FROM	
	Intra-auricular pressure	Jugular pulse
	second	*second*
C84	0.14	0.131
C88	0.13	0.096
C89	0.05	0.028
C91	0.038	0.048
C125	0.075	0.060
Average	0.087	0.0726

Since the rise of the presystolic wave very nearly equals the dynamic interval, the duration of the A_s-V_s interval may be approximated by the *a-c* interval. Unfortunately, this is of little scientific value because the A_s-V_s interval is not proportional to the intersystolic interval and its relation to the conduction time is questionable. The writer has elsewhere (6) discussed the assumption that must be taken for granted, especially in pathological cases when the A_s-V_s interval is used as a criterion of conduction time.

Relation to the waves of the electrocardiogram lead II. The relations of the intra-auricular pressure variations to the waves of the electrocardiogram taken by lead II have been recently reported by Garten and Weber (7). Their results will be referred to in comparison with those yielded by a consideration of nine experiments in which the auricular myogram was also recorded. Figure 3 shows such a record. The first electrical variation starts at 1. It precedes the rise of intra-auric-

ular pressure, 2, by an interval of 0.022 second and the onset of mechanical shortening, 3, by 0.033 second. The former figure was duplicated or approximated in other experiments (cf. figs. 4 and 5) and agree with the results of Garten and Weber who report variations from 0.013 to 0.021 second.

The precise relation of the onset of the intra-auricular pressure rise to the *P* wave varies slightly. It may, as shown in figure 3, occur precisely at the summit of the *P* wave. This also accords with the tracings published by Garten and Weber. It was found more frequently, as shown in figures 4 and 5, to come somewhat earlier. While there is no precise relation between the mechanical systole and the *P* wave of the electrocardiogram, the summit of the *P* wave may, without great error, be taken as indicating the approximate onset of auricular systole.

The relation between the end of mechanical systole and the waves of the electrocardiogram is still more variable. In figure 3, auricular systole does not terminate until the completion of the *R* wave, 4, and this is followed after a short interdynamic interval, 5, by ventricular systole, as indicated both by the heart sounds and intra-auricular pressure curve. This, it may be emphasized, proved later than the average cases in which, as shown in figures 4 and 5, it terminated just before or after the peak of the *R* wave. According to these results, auricular systole does not terminate until ventricular excitation is well under way.

III. PHYSIOLOGICAL IMPORTANCE OF RESULTS

The data collected in this and the two preceding papers enables us to state with greater precision the details of the cardiac cycle.

About 0.02 second after an impulse is emitted from the sino-auricular node the right auricle begins to contract, as evidenced by the rise of intra-auricular pressure. The precise onset of this mechanical snortening can not be determined from the waves of the venous pulse but may be approximated by the peak of the *P* wave taken by lead II. This mechanical shortening is due to a series of fractionate contractions which spread across the auricle in the wake of the excitation wave. The auricle continues to shorten as long as the contracting units of cardiac tissue over-balance the relaxing units. As soon as an exact balance is established no further shortening is possible and the termination of the systole has come. This period lasts about 0.11

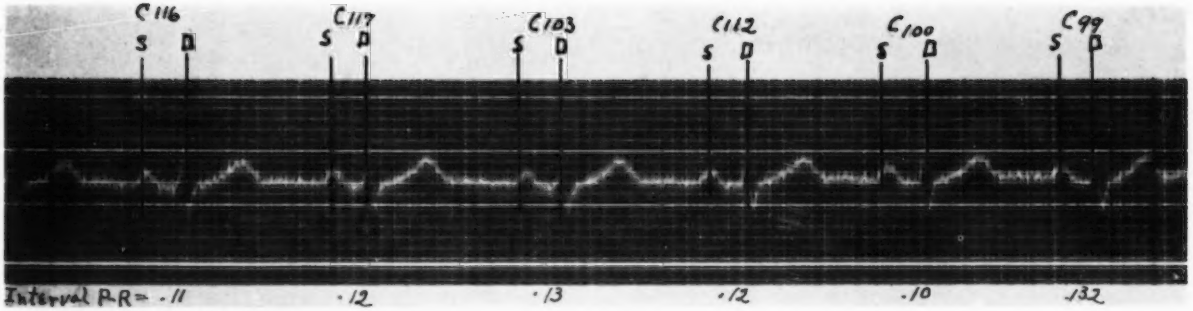

Fig. 5. A normal electrocardiogram, lead II, upon which have been marked for comparison the beginning (*S*) and end of auricular systole (*D*) as found in different experiments. At bottom, *P-R* intervals found in these cases are marked. Time, 0.05 second.

second. The contraction of the auricle exerts a dynamic action by elevating intra-auricular pressure. This occurs early in systole but continues for only 0.05 second; during the remainder of systole the intra-auricular pressure falls. This *dynamic interval*, as the period during which pressure rises is called, may be estimated from the rise of the presystolic wave of the venous pulse, *a*-wave, but the total duration of systole can be determined neither from the venous record nor the electrocardiogram.

The auricle continues to contract after excitation of the ventricle has occurred, as shown by the fact that it terminates during the *R* variation of the electrocardiogram. The ventricle normally never contracts until auricular systole is completed. Occasionally ventricular systole follows immediately but usually an intersystolic interval, averaging 0.024 second, intervenes. The duration of this interval can be accurately determined neither from the jugular tracing nor from the electrocardiogram. The *a-c* interval of the jugular pulse in normal and regular rhythms very nearly equals or at least varies with the A_s-S_s interval, but the latter is not a criterion of the intersystolic interval and does not vary with it.

It is evident from this work and that of previous investigators that the electrocardiogram is capable of showing (1) the time of emission of an impulse from the sinus node (P wave); (2) approximately the onset of auricular systole (summit of *P* wave) and (3) the conduction time between sinus and ventricle (*P-R* interval). The venous pulse gives neither the onset of auricular systole nor its duration but the dynamic interval of systole may be calculated from the rise of the presystolic wave.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VII. Variations in the Chemical Composition of Reproductive Tissues in Relation to Variations in Functional Activity

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Tissues in which prolonged periods of great activity alternate with long periods of nearly complete rest are unusual in animals. Fully differentiated and functionally active tissues which undergo a permanent and sudden change from a very low state of activity to a much higher one are also uncommon in animals. Some of the reproductive tissues of birds are admirably adapted to securing tissue samples in which this contrast is most pronounced.[1] Such contrasting states of the tissue may here be obtained moreover without the least artificial stimulation or restriction of any of the habits, powers, or processes of the animal. The chemical differences observed in tissues so obtained should therefore be of considerable interest.

Mayer and Schaeffer (1) found that the tissues of some hibernating animals show seasonal fluctuations in their content of lipoid phosphorus. And, further, when the body temperature of warm-blooded animals is

[1] A fact deserving consideration is that in animals the reproductive system, of all the systems of the organism, is the most easily, and most profoundly affected by environmental and intra-organic change. Darwin made the following observation: "Many facts clearly show how eminently susceptible the reproductive system is to very slight changes in the surrounding conditions. Nothing is more easy than to tame an animal, and few things more difficult than to get it to breed freely under confinement, even when the male and female unite." (Origin of Species.) This fact then also invites a comparison of analytical data from these tissues in their functional and non-functional states. The further fact that the *time relations* of ovulation, and of other activities of the reproductive organs, are easily ascertained in birds—much more easily than in either mammals or reptiles—is an additional and valuable asset in the prosecution of such studies.

either raised or lowered to proper levels, the lipoid phosphorus shows marked variations, and these variations by no means always parallel the variation in total fatty acids. Finally, the work of Mayer and Schaeffer would seem, in the main, to indicate that *changes* in the physiological activity of the tissues (lungs, liver) studied are, in a majority of cases, associated with an *increase* of phosphatides in the tissue. The probable rôle of the phosphatides of the cell has recently been rather fully treated by Mathews (2, chapters 3 and 13).

The present studies deal with two general types of tissue:

(1) The follicular membrane, the immediate envelope of the growing ovum. This membrane, from ovarian ova of the fowl, was obtained at various stages of its activity, two stages of which have been shown—in the first of this series of studies (3)—to contrast as the figures 1: 25.8 in the rate at which the constituents of yolk pass through this membrane.

(2) The oviduct of the fowl, which was always divided for analysis into two of its functionally differentiated parts; (a) the lower (posterior) shell secreting gland or uterus; and (b) the upper (anterior) albumen secreting portion. The functioning glands (oviducts containing eggs) are contrasted with oviducts free from the presence of eggs (i.e., without the efficient stimulus to the secretory activity of these glands) for longer and shorter periods.

Our analyses of the follicular membranes were made in 1910–1911. The results of these analyses give some support to the view that a *change* in rate of activity, in the direction of greater activity of the tissue, is accompanied by an increased phosphatide content of the tissue; but, after the change in activity is effected, the percentage of phosphatides is little, if at all, higher than in the period which preceded the change. Our evidence on these points would be much strengthened if the per cent of solids in this tissue had proved to be larger, or if the preparation of larger samples had not been discouraged by the excessive tediousness of the process of freeing and cleaning these minute membranes.

The phosphorus values obtained from the albumen secreting portion of the oviduct clearly show that the resting gland (i.e., one that has not functioned for three or four days, and will not again function for a similar period), contains a higher percentage of alcohol-ether soluble phosphorus than does the same gland a few hours before, or a few hours after, the actual discharge of albumen. The alcohol-ether insoluble phosphorus also seems, in a much less pronounced way, to follow the same rule. The several facts obtained on the composition of the

various tissues studied, and on the relation of rest and activity to variations in composition, will later be considered in detail.

METHODS OF PREPARATION AND ANALYSIS

Analysis. The methods of analysis used with the *follicular membranes* are those fully described[2] by Koch (4), and are the same as were used and briefly described in the several previous papers of this series of studies. This includes a four-hour extraction with alcohol, followed by a one-hour extraction with ether, and this by another twelve-hour alcohol extraction in a Koch apparatus. The alcohol-soluble, water-insoluble phosphorus was determined by the Neumann-Pemberton method and in all of the tabulated data was calculated (no. cc. of $\frac{N}{2}$ NaOH used x 0.6742 mgm.; then this figure x 25.75) as lecithin (phosphatides). Other phosphorus than lipoid phosphorus forms a part of the figures for ash.

The analysis of the *oviducal tissues* was shortened (extraction same as before) to estimations of moisture, alcohol-soluble and alcohol-insoluble fractions, and to phosphorus determinations on the whole of each of these two fractions. The phosphorus of the oviduct was calculated as P_2O_5.

Preparation. The follicular membranes, of the several sizes, were obtained from the ovaries of laying hens. The ovaries were taken immediately from the just-killed hens. The dimensions given for the membranes are those of the diameters of the ovarian ova which they surrounded.

The preparation of the four samples under *a* and *b* was an extraordinarily tedious task. The second sample under *a* for example required the use of 1020 follicles. The eggs of appropriate size were stripped of the *thecae* with fine forceps; the remaining follicular membrane was then slit open, over about one-half of its circumference with fine scissors, and all traces of yolk quickly washed from the interior with distilled water. The immersed membrane in water tends to retain or re-assume its round shape, and after a few light rapid strokes with a smooth blunt instrument it becomes perfectly transparent and clean. It is then dried to approximately normal weight on filter paper. Unfortunately these membranes proved to have a lower per cent of solids than was expected; and since the labor of obtaining the clean membranes was excessive, the size of many or most of these samples proved to be

[2] The sulphur determinations are omitted in all our analyses.

smaller than is desirable. The figures obtained from the smallest samples are probably not wholly reliable even for the first two (alcohol-soluble and alcohol-insoluble) fractions obtained.

Oviducts were obtained in varying stages of rest and activity. Each complete oviduct (less the vaginal portion) was divided at the line of the isthmus into the upper albumen secreting portion (plus the funnel) and the lower shell gland (plus the isthmus, which secretes the shell membrane). Care was taken not to include in the samples of shell gland any of the fat which often overlies the vagina and sometimes extends upon the shell gland. The oviducts were taken fresh and warm from hens whose eggs had been trap-nested. The fresh samples were weighed, thoroughly crushed in a porcelain crucible and preserved for two to four weeks before analysis in large quantities of 95 per cent alcohol.

THE COMPOSITION OF THE FOLLICULAR MEMBRANE

The two analyses under *a* of table 1 are of membranes from the slow-growing ova of 3 to 4.5 and 3 to 5.5 mm. diameter. The ova enveloped by these membranes contain only white yolk, and this yolk, as noted above, passes through the membrane at about one twenty-fifth of the rate at which yolk later passes through these membranes (3). The data from these two analyses indicate that in this young and relatively inactive stage the membrane contains less phosphorus than in stage *b*, the stage in which the shift to a greatly increased transfer of yolk material occurs. The amount of phosphorus in these smallest membranes is, however, nearly equivalent to the phosphorus content of the much larger membranes *after* the period of increased activity is established (d and dd).

The two analyses (b) of membranes taken closest to, and including, the period of an enormous increase in the passage of yolk materials through the membrane differentiate themselves from all of the other analyses in showing extremely high phosphatide values. One of the two samples yielded a very high alcohol-soluble fraction (27.46), but the other (15.98) is not above the average in this respect. It seems probable that these two samples which yielded the highest phosphorus values contained the smallest amounts of neutral fat. The data certainly indicate that the membranes at this the period of *change* in degree of activity contained least neutral fat in propprtion to phosphorus soluble in alcohol and insoluble in water. These analyses therefore indicate that at the period of a *change* in the activity of these

TABLE 1

*The composition of the follicular membrane of the fowl at various stages of its growth and activity**

	NATURE OF MEMBRANE	WEIGHT OF SAMPLE	IN PER CENT OF SOLIDS							H_2O
			Alcohol soluble	Alcohol insoluble	Phosphatides† (P as lecithin)	Neutral fat	Protein	Organic extractives‡	Ash‡	
a	3–4.5 mm. follicular membranes (432).....	1.489	17.71	82.29	9.77	6.10	74.66	4.92	4.55	85.21?
	3–5.5 mm. follicular membranes (1020)...	2.188	14.15	85.85	5.13	7.06	76.35	7.54	1.92	84.05?
b	5–6 mm. follicular membranes (223).....	1.624	15.98	84.02	14.00	traces	76.86	4.18	5.01	85.74?
	4–6.5 mm. follicular membranes (no. ?)..	1–2g.?	27.46	72.54	23.10	traces	72.54	2.58	2.58§	
c	5–13 mm. follicular membranes (316).....	2.236	13.72	86.28	3.65‖	7.29	78.42	8.36	2.29	84.18?
d	15–34 mm. follicular membranes (25).....	3.645	14.75	85.25	13.10		77.45	7.94	1.51	87.20?
	18–34 mm. follicular membranes (14).....	2.880	18.02	81.98	8.55	7.05	76.12	4.39	3.88	88.06?
dd	Inner capsules (12) of largest ova; outer layer removed.....	3.110	19.36	80.65	10.27	6.47	75.95	3.59	3.72	87.88?
e	Entire follicles (5) of liberated ova.....	1.415	36.18	63.82	12.99	19.93	57.88	3.94	5.26	80.50?

* We are indebted to Miss A. A. Spohn for assistance with this series of analyses.

† The alcohol-ether soluble, water insoluble phosphorus is alone represented here, and is calculated (P x 25.75) as lecithin.

‡ The smallest samples do not permit accurate determinations of extractives and ash; the figures are too high in most cases. Only the data from the larger samples are approximately correct.

§ Organic extractives and ash were here determined on the alcohol-soluble substance only; the *protein* value is therefore too high; the value is that of the entire alcohol-insoluble fraction.

‖ The lecithins here were incompletely precipitated; the figure for phosphorus is therefore too low and the figure for fat is correspondingly too high.

membranes (to a much higher activity), the phosphatide content of the tissue was increased, and the neutral fat content was diminished.

These data are of further interest in connection with the Overton theory of the permeability of membranes. They suggest that among the lipoids the phospholipins play the chief part in a greatly increased permeability of membranes. The fact of an enormous increase in the rate of transference of substances through this membrane at this period (5 to 6 mm.) is firmly established (3); at this point this membrane increases by about twenty-five times its previous rate of passage (or secretion?) of yolk constituents to the ovum. At this same period the ratio of phosphatides to neutral fat is by far the most extreme found in the entire series of analyses. It may be, of course, that in this period of the growth and activity of this tissue an unusual percentage of alcohol-ether-soluble and water insoluble phosphorus exists in molecules much smaller than that of lecithin, and but little in the form of lecithin. This point has not been investigated. The data indicate, however, that the amount of phosphorus having the solubilities just noted, in relation to neutral fat, is much higher than that usually found in other stages of the existence and activity of this tissue.[3]

That the unusually high proportion of phosphatides of analyses *a* to *d* are not to be accounted for by contamination of the membranous tissue with yolk is shown by the fact (5) that in the egg yolk of the fowl the ratio of phosphatides to fats (analyses and calculation the same) is 11:25 (yellow yolk), and 11:23 (white yolk). The substance that passes through all these membranes is therefore much richer in neutral fat than in lecithin; but the membrane through which these substances pass, at one important stage at least, contains these substances in wholly reversed proportions.

The membranes composing analysis "dd" are not wholly comparable with the preceding membranes, since only the theca externa, bearing the large blood vessels, was stripped from these nearly mature follicles; leaving here both the theca interna and follicular membrane. The above-mentioned ratio of phosphatides to fat obtains also in this tissue.

[3] In analysis *c* some membranes from the period of *changing* permeability (5 to 6.5 mm.) were included, and the values found in this analysis should be intermediates of those for b and d. Apparently they are not. The incomplete precipitation of lipoid phosphorus, and the unknown proportions of smaller and larger membranes in the sample, make the value of the analysis somewhat uncertain.

This relation does not persist, however, in the (entire) follicles or capsules which have completed and entirely passed their functional period (analysis e). In this stage phosphatides are to the fats as about 13:20. This is, of course, a degenerating tissue, and on this basis its high fat content is to be expected. If, however, there exists in birds a tissue comparable with the *corpus luteum* of mammals, an improbable supposition, then that tissue should be included in this material, the degenerating follicle. It may be noted that the amounts of water, fat and protein in this tissue depart from the corresponding amounts in all of the preceding tissues, and in the direction of the amounts characteristically found in mammalian luteal tissue.

Several of the analyses were made on smaller amounts of tissue than is desirable, because as already noted, the preparation of the smaller membranes is an extremely tedious task and requires the sacrifice of many animals. The figures obtained for ash and organic extractives from the smallest of these samples are probably too high. Even in the more adequate samples, however, the percentage of water-soluble organic substance is notably high.

The moisture figures on the series of membranes is of interest. Although considerable amounts of connective tissues are present in all these membranes the water content ranges from 80.5 per cent to 88.0 per cent. We do not believe that these samples as prepared and weighed departed at all widely from their normal state, though the possibility for increase or decrease was present in all cases. In the table the accuracy of all moisture figures is questioned.

THE COMPOSITION OF THE OVIDUCAL TISSUES

The shell gland. In table 2 the oviducal tissues are arranged in order of their activity; or better, the shell glands are arranged in the order of imminence of, or readiness for, their period of secretory activity. The glands that would soonest have come into full activity are placed in the top rows of the table.

Noting first the phosphorus values it will be seen that the three oviducts (122, 120, 126) which were ready for their highest activity do not consistently differ in the amount of alcohol-ether soluble phosphorus from the inactive glands (124, 130, 128). On the other hand, the alcohol-insoluble phosphorus seems to be present in somewhat greater amount in the active than in the inactive glands. The total of alcohol-ether soluble substance is appreciably less in the active than in the inactive glands.

TABLE 2

Analyses of functioning and non-functioning shell glands and upper (albumen-secreting) oviducts of the fowl

ANALYSIS	TISSUE	POSITION OF EGG	WEIGHT	WATER	PER CENT MOIST WEIGHT			PER CENT DRY WEIGHT				
					Alcohol soluble	Alcohol insoluble	P_2O_5	Alcohol soluble	Alcohol insoluble	Total P_2O_5	Alcohol soluble P_2O_5	Alcohol insoluble P_2O_5
122	Shell gland	With shell membrane; entering shell gland, (funnel also gripping ovarian egg)	13.92	83.56	3.62	12.92	0.39	21.87	78.13	2.35	3.31	2.08
123	Upper oviduct		30.70	77.72	4.04	18.23	0.20	18.14	81.86	0.91	0.77	0.95
120	Shell gland	With membrane; entering shell gland	17.76	83.88	3.97	12.15	0.32	24.64	75.36	1.97	2.51	1.79
121	Upper oviduct		27.45	79.94	4.26	15.81	0.12	21.23	78.77	0.61	0.88	0.53
126	Shell gland	With shell membrane; 18 mm. above shell gland	11.40	83.81	3.88	12.30	0.44	23.99	76.01	2.74	4.42	2.21
127	Upper oviduct		20.06	77.45	4.59	17.96		20.34	79.66			1.26
124	Shell gland	None: egg laid same day.*	16.60	83.59	4.53	11.88	0.25	27.61	72.39	1.53	1.68	1.48
125	Upper oviduct		27.37	74.33	3.75	21.92	0.28	16.62	85.38	1.09	2.72	0.81
130	Shell gland	None: last egg 3 days earlier†	6.29	80.86	4.68	14.46	0.46	24.45	75.55	2.41	4.71	1.64
131	Upper oviduct		7.81	76.35	5.11	18.54	0.55	21.60	78.40	2.34	4.79	1.66
128	Shell gland	None; last egg 4 days earlier‡.	13.27	81.33	5.75	12.90	0.40	30.82	69.18	2.16	3.01	1.78
129	Upper oviduct		20.42	77.18	5.76	17.06	0.39	25.25	74.75	1.73	3.58	1.10

* An egg in ovary ready to ovulate; would have entered oviduct within a few hours.

† Largest egg in ovary = 17 x 13 mm.; would probably have entered oviduct about three or four days later.

‡ Largest egg in ovary = 14 x 13 mm.; would probably have entered oviduct about four days later.

It is of interest to note that the moisture values obtained from the four shell glands which were ready to function, or had just ceased to function (124), are high and quite nearly equivalent, ranging from 83.46 per cent to 83.88 per cent; while the quite inactive glands gave notably lower values (80.86 and 81.33). It seems not improbable that this incidence of low moisture and tissue inactivity is a fact of importance. The analyses of upper oviducts also show, in the main, a similar association of lower moisture values in the more inactive state of the tissues.[4]

The upper oviduct. The alcohol-ether soluble phosphorus of the active albumen glands (which had ceased their active secretion of albumen only a few hours previous to the taking of the sample and would, within a few hours, again have become active) is small in amount; it is perceptibly increased in those glands which had been inactive, and would certainly have remained inactive, for longer periods. The total phosphorus of the upper oviduct is usually considerably smaller than the percentage found in the shell gland, and this situation is, no doubt, connected with the fact that the secretion product of the upper oviduct—the egg albumen—contains only traces of phosphorus, while in the egg-shell this element is abundant. The alcohol-ether insoluble phosphorus of the upper oviduct apparently does not vary consistently in this series. Probably the total alcohol-ether soluble substance is increased in the inactive state of the glands.

The moisture determinations on these albumen secreting glands offer some evidence that the less active glands contain least water, a situation to which reference was made above.

[4] Undoubtedly a somewhat higher percentage of blood is present in the active than in the inactive glands, and fowls' blood contains about 86 per cent of water. But in order to raise the water content of the inactive shell gland no. 128 to the average value found for the active glands the former would have to receive an additional quantity of blood equal to its total inactive weight—and this without the addition of any new tissue with less than 86 per cent water. This demonstrates that the higher water values of the active glands cannot be attributed to increased vascularity alone.

On the other hand, it is not improbable that the differences observed in the water content of the active and inactive *upper oviducts* is ascribable to an influx of blood to the active gland. The differences are here smaller in amount, and, owing to the low water content of this tissue, each added gram of blood would here much more greatly affect the percentage of water in the analyzed tissue.

The very small size of the oviduct that furnished analyses 130 and 131 is only partly to be accounted for by shrinkage of tissue under inactivity. The hen from which this oviduct was taken was much smaller than the other hens (all mongrels) killed for this purpose.

Comparing the chemical composition of the upper and lower parts of the oviduct one first notes that the shell gland always has a considerably higher water content than that of the upper oviduct with which it was connected. This brings to light the interesting fact that that part of the oviduct whose secretion is about 90 per cent water, is itself relatively water poor; while the other part, whose secretion is more than 95 per cent solids,[5] is relatively water rich.

Perhaps the question should be asked, Is an albumen secreting gland, or a shell gland, really active (or predominatingly active) only when it is actually discharging its secretion? Are such of these glands as are called upon during a few hours each day, in fowls laying daily, really quiescent during the intervals between discharges of secretion? May not such intervals be the truly active, reconstructive, or preparatory periods? To these questions there is at present no definite answer. There can be no question, however, that the first eight analyses of table 2 represent more active tissues than do the last four analyses of the table. Oviducts completely free from eggs for three or four days, with no possibility of discharging a secretion during the succeeding three or four days, are unquestionably less active glands than those containing eggs and actually engaged, daily or almost daily, in the copious and rapid delivery of their secretory products.

SUMMARY

The membranes (membrana follicularis) which immediately surround the growing oöcytes (of 3 to 34 mm.) of the fowl were subdivided for analysis into (size) groups. Through the membranes (3 to 5.5 mm.) of group *a* yolk constituents are passed extremely slowly to the egg; group *b* includes those membranes (5 to 6.5 mm.) which suddenly, in a day, *change* the former rate of passage of yolk constituents from the blood to the yolk, to a rate about twenty-five times greater; group *d* includes membranes of the largest size (15 to 34 mm.); through these the constituents of yolk are passed or secreted at the rapid, but changeless, rate.

The phosphatides of all three groups of membranes probably exist in amounts relatively large in proportion to the neutral fats.

The greatest disproportion of phosphatides to neutral fats, and by

[5] One statement puts the moisture of the shell at about 1 per cent. In the state in which it is found in the uterus or shell gland the moisture of the shell is certainly considerably higher.

far the largest amounts of phosphatides, apparently are found in group *b*; it is at this stage of the growth or history of the follicular membrane that there occurs a most striking *change* in its permeability. Neither the disproportion of phosphatide to neutral fat, not the high phosphatide value persists under the new rate of permeability, i.e., *after* this new rate is established.

The results lend some support to the view that *changes*, at least a change toward greatly increased activity, in the physiological activity of a tissue is accompanied by an increase in the phosphatides of the tissue. Similarly, the results supply one case in which a change in the permeability of a membrane, toward increased permeability, is apparently accompanied by a perceptible increase in the phosphatide content of the membrane. According to one's classification of the follicular membrane as an active secretory, assimilative tissue, or as a membrane—a more or less passive filter for yolk constituents—the results apply to the one or the other of the above-mentioned problems. Admittedly, however, our results on this topic would be more conclusive if based on larger samples.

Active and relatively inactive oviducts, divided into shell glands and albumen secreting glands, of the fowl were analyzed, and their chemical differences noted.

The alcohol-ether soluble phosphorus of the shell gland (uterus) does not differ consistently in the active and inactive glands. Probably the alcohol-ether insoluble phosphorus is higher in the active glands. The total alcohol-ether soluble substance is greater in the inactive glands. The amount of moisture is plainly and significantly greater in the active glands.

In the albumen secreting glands the alcohol-ether soluble phosphorus is much increased under inactivity. It seems not at all possible to ascribe this increase to tissue shrinkage (or relatively greater loss of other constituents) under inactivity; the absolute amounts of phosphorus are increased, and this in a tissue whose inactivity is beyond question. This result for this gland is in contradiction to the results from the follicular membranes, unless most of the phosphorus of the inactive albumen gland is water soluble; and unless most of that of the active gland is water insoluble. This point remains undetermined. The percentage of water is somewhat larger in the active than in the inactive albumen secreting glands.

The shell glands secrete a substance containing very little water; the upper oviducts secrete a substance very rich in water; but the

shell glands, in both the active and inactive condition, contain 4 to 9 per cent more water than do the albumen secreting glands.

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A STUDY OF THE CONDITIONS INVOLVED IN THE ACCUMULATION OF DISSOLVED SUBSTANCES IN THE BLOOD

I. The Relation of Acidity to the Retention of Sugar and of Urea by the Colloids of the Blood and the Kidney

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A considerable number of special studies has been made concerning the conditions involved in the excretion of water by the kidney. It is evident that several factors enter into the process. It is necessary for "free" water (i.e., water not in colloid combination) to be present in the blood, and it is necessary for the kidney to be in condition to excrete it. Under various conditions the colloids of the blood may have more affinity for the water than the kidney tissue, whereas under other conditions, the colloids have less power to hold the water and some of it is set free. At times the kidney tissue may have abnormal retention powers. Fischer's (1) application of the fact that hydrophilic colloids retain more water, the more acid they are, and that the water content is to a certain extent a function of the total acid content (modified, in the case of different acids, by the nature of the anion radical), serves as a simple description of the various kidney states. A more "acid" kidney will tend to retain more water than one in a less acid condition. Excretion will then be reduced. Do similar conditions affect the diffusion of urea, uric acid, creatinine, glucose, chlorides, or other dissolved substances?

PART I

In a previous paper (2) one of us showed that phenolsulphonephthalein is retained by the hydrophilic colloids of blood serum in proportion to the acid content of the serum.

TABLE 1

SOLUTION	$\frac{N}{10}$ HCl ADDED TO SOLUTION	DEPTHS OF COLUMNS OF LIQUIDS TO GIVE SAME COLOR
	cc.	*mm.*
3 cc. sheep blood serum, 2 cc. 0.8 per cent NaCl, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein		21
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum		17
3 cc. sheep blood serum, 1 cc. 0.8 per cent NaCl, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein	1	47
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum	1	17
3 cc. sheep blood serum, 0.3 cc. 0.6 per cent aqueous solution phenolsulphonephthalein	2	90
Control: Same, but with 3 cc. 0.8 per cent NaCl instead of the blood serum	2	17

In those experiments, the results of which are given in table 1, 3 cc. of each solution, after a thorough mixing, were placed in parlodion sacs prepared after the method of Levy, Rowntree, and Marriott (3). The sacs were lowered into an 0.8 per cent sodium chloride solution until the level of the surrounding solution was the same as that in the sac. Diffusion was allowed to proceed for fifteen minutes, and the sac was then removed and rinsed off with distilled water. The salt solution with the rinsing water was made alkaline by the addition of 10 cc. of 10 per cent sodium hydroxide, and distilled water was added until the volume was 100 cc. The varying shades of pink showed that different amounts of phenolsulphonephthalein had diffused through the walls of the tubes, making a quantitative determination of the dye possible by means of the Duboscq colorimeter. The table shows that as the acid content of a lyophilic colloid (the blood serum) is increased, there is a corresponding retention of the dye, as shown by the lessened diffusion.

Blood serum, however, does not show this behavior toward all dissolved substances. The first group of experiments below, show that in the case of urea and glucose, the blood serum does not retain these substances.

In the following experiments, blood serum with known amounts of acid and of glucose was placed in parlodion sacs, and the contents allowed to diffuse into an 0.8 per cent sodium chloride solution. The technique is described above.

The following protocol will serve to show the results. Three cubic centimeters of the solutions were allowed to diffuse for thirty minutes, and after dilution to 100 cc., the amount of sugar was determined by Benedict's method. (Results expressed in terms of cubic centimeters of the 100 cc. used to reduce 25 cc. of Benedict's solution.)

TABLE 2

SOLUTION	$\frac{N}{10}$ HCl ADDED TO SOLUTION	DIFFUSED SOLUTION OF GLUCOSE TO REDUCE 25 CC. OF BENEDICT'S REAGENT
	cc.	*cc.*
10 cc. blood serum, 5 cc. 25 per cent glucose, 2 cc. 0.8 per cent NaCl		25.25 24.55
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum		23.60
10 cc. blood serum, 5 cc. 25 per cent glucose, 1 cc. 0.8 per cent NaCl	1	24.40
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum	1	22.20
10 cc. blood serum, 5 cc. 25 per cent glucose	2	24.50
Same, but with 10 cc. 0.8 per cent NaCl solution instead of serum	2	22.20

This experiment indicates that there is no corresponding variation in glucose retention by the colloids of the serum under variations in both total acid content as well as hydrogen ion content.

A similar series of experiments were conducted using urea solutions in the parlodion sacs. The urea which diffused out in thirty minutes was determined by Benedict's method. The results are shown in table 3.

The table shows that variations in acidity were not accompanied by any corresponding change in the urea retention. It is therefore evident that, since with an increased acid content, the serum shows an increased affinity for phenolsulphonephthalein, but does not show a corresponding behavior towards glucose and urea, the colloids of the

blood must exert a *selective adsorption* for some substances and not others. The practical significance of this will be taken up in the general discussion of results.

TABLE 3

SOLUTION	$\frac{N}{10}$ HCl ADDED	UREA IN DIFFUSED SOLUTION
	cc.	*per cent*
10 cc. blood serum, 10 cc. 18 per cent urea, 4 cc. 0.8 per cent NaCl		0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)		0.375
10 cc. blood serum, 10 cc. 18 per cent urea, 2 cc. 0.8 per cent NaCl	2	0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)	2	0.405
10 cc. blood serum, 10 cc. 18 per cent urea	4	0.366
Same, but with 0.8 per cent NaCl instead of serum. (10 cc.)	4	0.375

PART II.

Under certain pathological conditions, sugar or urea accumulate in the blood. The experiments in Part I indicate that the accumulation cannot be due to retention by the colloids of the serum, at least on a basis of acid content. May this be due to faulty powers of elimination? We must therefore note the behavior of the kidney colloids toward dissolved substances under various conditions.

While some phases of the study of the retention of substances by blood serum can be satisfactorily investigated by means of the parlodion sac diffusion method, it is necessary to use other means in studying the properties of the kidney colloids. For this purpose we have found that the perfusion method described by Sollmann (4) is admirably adapted to our needs. By this procedure we were able to throw some light on several phases of secretion and retention of both water and dissolved substances by the colloids of the kidney. Briefly the technique is as follows:

In anaesthetized rabbits, cannulas are tied in the renal artery and vein, and in the ureter. The apparatus, as described by Sollmann, is arranged for the perfusion, under uniform pressure, of salt solutions

through the kidney. After washing out all the blood with either 0.9 per cent NaCl solution, or 0.9 per cent NaCl and 0.2 per cent to 0.5 per cent glycocol, both solutions being made alkaline (pH = 7.3–7.6), the kidney was removed from the body, mounted on a glass plate in a holder, and evaporation prevented by a covering of cotton, which was wet with 0.9 per cent NaCl made alkaline to pH = 7.8–8.0 with NaOH.

Solutions were then prepared containing 0.9 per cent NaCl and 0.5 per cent urea, and the hydrogen ion concentration was varied with traces of hydrochloric or phosphoric acid, or sodium hydroxide. We then had solutions of practically the same composition, but with different hydrogen ion concentrations. The concentrations used in these experiments were pH = 6.6, 6.8, 6.9, 7.0, 7.2, 7.3, 7.4, 7.5, 7.6, 7.8, 7.9, and 8.0. It will be noticed this range, extending from the acid pH = 6.6 to the alkaline pH = 8.0, covers a series which is well within physiological limits. The variations between the solutions were extremely delicate, and represented gradations that could easily be brought about in the blood of the body during rest, fatigue, feeding, fasting, anaesthesia with chloroform or ether, emotion, or during disease. Besides these solutions, others were used in which the concentrations of the constituents were varied.

The procedure used for any one kidney was to pass the solutions—50 to 150 cc. from an arterial reservoir—into the artery, and collect the vein fluid and the ureter fluid. After passing one solution through, a second, third, or fourth solution followed, the vein and ureter fluids being collected separately. Thus we were able to start with an alkaline solution, and follow it with ones more acid, or use the reverse order. Oxygenation was accomplished by allowing air to bubble through the solution in the arterial reservoir. In each case the hydrogen ion concentration of the artery solution was taken from a specimen drawn from a valve, just before it entered the renal artery. The method used was that of Levy, Rowntree, and Marriott (3). The determinations in all cases were made immediately after collection of the liquid. The experiments covered periods of from fifteen minutes to two hours, varying with the number of solutions passed through. In each case the venous solution was more acid than that of the artery, and the ureter fluid more acid than that of the vein, showing that acids and CO_2 were being eliminated, having arisen from autolysis and oxidation. The amount of urea was constant in the artery, so that variations in excretion of urea could not be due to the variation of supply.

The results, tabulated below (tables 4, 5, 6, and 7), will be discussed under the following headings:

a. Relation of urea excretion to the acidity of the arterial fluid.

b. Relation of acidity of the arterial fluid to the retention of urea by the kidney colloids.

c. Relation of water excretion to the acidity of the arterial fluid.

TABLE 4

Experiment 4. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY pH	UREA IN ARTERY FLUID	UREA IN VEIN FLUID	UREA IN URETER FLUID	$\frac{V+U^*}{A}$
	per cent	*per cent*	*per cent*	
7.3	0.5	0.399	0.288	0.687: 1.0
6.6	0.5	0.405	0.208	0.613: 1.0
7.6	0.5	0.411	0.411	0.822: 1.0

* V = per cent of urea in vein fluid.
U = per cent of urea in ureter fluid.
A = per cent of urea in artery fluid.

TABLE 5

Experiment 5. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY pH	UREA IN ARTERY FLUID	UREA IN VEIN FLUID	UREA IN URETER FLUID	$\frac{V+U}{A}$
	per cent	*per cent*	*per cent*	
7.0	0.5	0.430	0.330	0.76: 1.0
7.2	0.5	0.370	0.440	0.81: 1.0
7.5	0.5	0.462	0.459	0.92: 1.0

TABLE 6

Experiment 6. Solutions passed successively through one kidney

HYDROGEN ION CONCENTRATION OF ARTERY FLUID pH	UREA IN ARTERY FLUID	UREA IN URETER FLUID
	per cent	*per cent*
7.3	0.5	0.348
7.5	0.5	0.438
8.0	0.5	0.471
7.8	0.5	0.462
7.7	0.5	0.422

All solutions listed in the order of their perfusion.

Taking into account the artificial conditions to which the kidney was subjected, the absence of nerve and vasomotor control, the difference between salt solution and blood, the differences in the animals at the time of operation, the disturbance in the oxygen supply, the results were fairly uniform, and warrant the conclusions given in the following pages.

A study of these results will show that the amount of urea excreted is independent, to some extent, of the order in which the arterial solutions were used. Thus in table 4, an alkaline solution was followed by an acid solution, and this again by one more alkaline. In table 5, the series is one of increasing alkalinity, while in table 6 the series becomes more alkaline, and is then replaced by ones more acid. The ureter fluids contained a different amount of urea than the vein, and, as the tables show, the amounts varied with the hydrogen ion content. The results show clearly that the *amount of urea excreted increased as the hydrogen ion concentration of the perfusing fluid decreased.* In other words, the less acid the arterial solution, the more urea was excreted.

The artery fluid in tables 4 and 5 contains 0.5 per cent urea. If the ureter fluids were merely an overflow from the blood, and not a "secretion"—selective, from a quantitative point of view, then we could expect a maximum of 0.5 per cent urea in the vein and 0.5 per cent urea in the ureter fluid. Their sum would be 1.0 per cent, and would equal twice that of the artery. The experiment shows that the sum falls short of this, that neither the ureter fluid nor the vein fluid reaches the strength of the artery fluid, and that furthermore, their combined strength falls short of the calculated amount. It is evident that some of the urea is retained by the kidney, and a glance at the tables shows that *the more acid the solution, the more urea is retained.* As the solution approaches pH = 7.6 or the normal H ion concentration of the rabbits' blood, less urea is retained. We would therefore conclude that the lack of secretion of urea in an acid condition of the body is due to its retention by the tissues of the kidney type rather than those of the blood serum, as shown in the first part of this paper. This finds interesting confirmation in the work of Long and Hull (6), who note that the kidney, on analysis, contains more urea than some of the other organs. Their animals were anaesthetized, and therefore they were dealing with kidney tissue at pH = 7.0, which is really "acid" for the rabbit. It therefore contained more urea than the other organs whose colloids probably were more of the type of those found in blood serum. We were able to wash out urea by perfusing a 0.9 per cent

salt solution through the kidney, if we passed an alkaline urea-free salt solution through an acid kidney. This suggests a physiological reason for the use of alkalies in conditions of urea accumulation.

We have, then, a mechanism for *selective adsorption* and *selective secretion* on a basis of differences in the nature of colloids, so that one type has greater adsorptive powers than another under different hydrogen ion concentrations. We would suggest giving the name *hylophilic* (hylo—"substance," referring to the solute) to the behavior of the kidney colloids towards urea, and *hylophobic* to the relationship of the serum colloids and perhaps some of the other body colloids to urea. In the end, we are dealing with the law of relative solubilities.

The kidney can take up urea while acid, and secrete it while less acid. The kidney can become less acid by the oxidation of acids, so that we would expect excretion to be a process which is accompanied by oxygen absorption by the kidney. The production of carbon dioxide was shown in our experiments, as the vein solution was always more acid than the artery fluid, and the ureter fluid more acid than the vein. However, the hydrogen ion concentration of the ureter fluid in these experiments was less than that of the acid urine of some types of nephritis, and in most cases the vein and ureter fluids were below pH = 6 and were therefore alkaline to methyl red. In some experiments, glycocol (0.2–0.5 per cent) was used to absorb the carbon dioxide formed, and the experiment showed that in every case, cloudy swelling of the kidney was long delayed beyond the time that it appeared when salt solutions alone were used. Although glycocol itself made the solution more acid, the hydrogen ion concentration was restored by the addition of sodium hydroxide, so that in the comparison, the glycocol and the salt solution were of the same degree of acidity.

It is significant that during and just after a meal, while there is more material for the production of urea circulating in the body, the blood and urine at the same time are more alkaline, and, if these results are in any way applicable to the living kidney, it would seem that the two factors work hand in hand to bring about a greater excretion of urea. On the other hand, in fasting, the conditions are just reversed, and a diminished urea production accompanies an acid condition of the blood and urine.

The next phase of the problem is, what is the relation between the hydrogen ion concentration of the artery fluid, and the amount of ureter fluid excreted? The experiments (table 6) show that *the lower the hydrogen ion concentration, the greater the secretion of water.* This

conclusion, we believe, bears out Fischer's (1) applications of the laws of colloid swelling under acidosis, to urinary secretions.

It will be noted that a very delicate change in the hydrogen ion concentration causes a marked change in the amount of water excreted. When the solution was too acid, as in experiment 4 (table 7), a toxic effect was exerted on the kidney. It showed a typical cloudy swelling—the picture of an acute nephritis, which it was. Protein was present in the vein fluid and in the ureter fluid, although the artery fluid was a salt solution, and contained no albumin.

TABLE 7

A separate kidney was used for each experiment

EXPERIMENT	COMPOSITION OF ARTERY FLUID	HYDROGEN ION CONCENTRATION OF ARTERY FLUID pH	URETER FLUID PER 100 CC. OF VEIN FLUID
			cc.
1	Ringer's solution, phosphoric acid (trace)	7.0	1.4
	Similar, but with 0.15 per cent urea	7.1	1.7
	Similar, with 0.15 per cent urea	7.3	4.2
	Similar, with 0.15 per cent urea	7.8	4.6
4	0.9 per cent NaCl; 0.5 per cent Glycocol	7.3	15.8
	0.9 per cent NaCl, 0.2 per cent glycocol; 0.5 per cent urea	7.3	23.22
	Same	6.6	8.05
	Same	7.6	6.28
6	0.9 per cent NaCl	7.3	13.55
	0.9 per cent NaCl; 0.5 per cent urea	7.3	21.03
	Same	7.5	27.39
	Same	8.0	33.75
	Same	7.8	25.80
	Same	7.7	15.10

The diuretic effect of urea, as shown in experiments 4 and 6, (table 7), is of considerable interest. The addition of 0.5 per cent urea to the perfusing solution causes an increase from 15.8 cc. of ureter fluid in experiment 4, to 23.32 cc., whereas in experiment 6, there is an increase from 13.55 cc. to 21.03 cc. Sollmann (3) reports that the diuretic effects of urea cannot be reproduced in the excised kidney of the dog and he attributes this to the fact that the kidney has lost its vitality, or that the blood plasma may have a counteracting effect.

The blood of rabbits normally varied in acidity around pH = 7.3–7.6 (i.e., alkaline) but after the anaesthetic it usually rises to a hydrogen ion concentration of pH = 7.0 (cf. Menten and Crile) (5). A solution, then, of pH = 7.0, while really neutral, is relatively acid for the tissues of the rabbit. *A normal salt solution or a solution of urea*, while usually pH = 7.0 or neutral in reaction, *is in reality "acid" to the tissues.* Such a fluid would naturally not cause an increased excretion, and Sollmann's experiment shows this to be true. If, however, as in our experiments, the acidity of this urea solution is decreased, and brought nearer to the normal for the body, a diuretic effect is noted. Chemi-

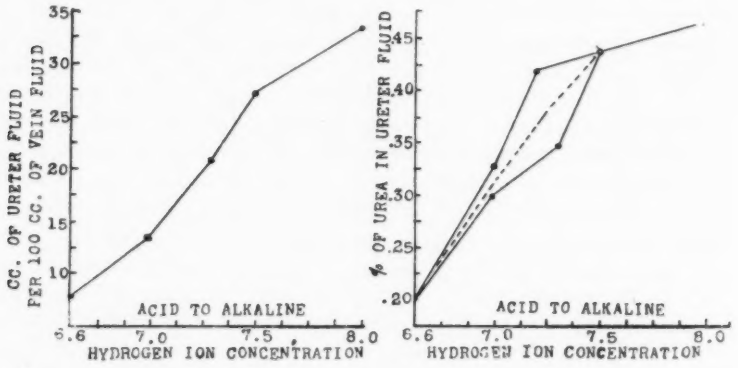

Fig. 1. The first curve shows the relation of the number of cubic centimeters of ureter fluid per 100 cc. of vein fluid to the hydrogen ion concentration of the artery fluid.

The second curve shows the relation of the percent of urea in the ureter fluid to the hydrogen ion concentration of the artery fluid.

The curves represent the mean of several experiments.

cally, urea acts as a base in the presence of acids, and tends to neutralize them. In the blood or body fluids, then, it would aid in keeping down the hydrogen ion concentration, an action which would explain its diuretic effects.

Two clinical observations bear out this point of view. After the administration of volatile anaesthetics, the secretion of urine is reduced, and often much delayed. In this condition we have the hydrogen ion concentration of the blood rising to pH = 7.0 or thereabouts, depending on the relative amount of the drug inspired, as Menten and

Crile have shown. Until the blood returns to its alkaline condition, the secretion of urine is lessened. It is further noted that after the intravenous injection of normal salt solution, the effects, while temporary, last for some time, (an hour or so) before the bulk is excreted. When we remember that the normal salt solution is pH = 7.0, and is therefore relatively acid, we can account for the delay in immediate excretion, both by the temporary "storage" of water in the serum colloids, and by the effect on the kidney.

While both the water and urea outputs increase with the decrease in acidity (see curve, fig. 1), nevertheless two isolated observations taken are of interest in suggesting that there may be a difference in the water and urea secreting mechanisms. In experiment 4, after the use of a very acid solution (pH = 6.6) an alkaline solution was unable to cause a total recovery in the water secreting mechanism, while there was an apparent recovery in the urea secreting mechanism. Furthermore, a kidney which had been kept in the ice-box over night, secreted water the next day, but very little urea. These suggest a subject for more detailed study.

At a given hydrogen ion concentration, only a certain amount of water or urea may be secreted, depending on the available free water or urea. If the supply of either is deficient, the excretion will be less, but the ability to secrete more will still exist. This means that from point of view of hydrogen ion concentration, the ability to excrete water is, in a measure, an index of the ability to excrete urea.

A study of the tables, especially experiments 4 and 6, will show that the previous solution influences, to some extent, the effect of the solution which follows. This is probably due to the extent of hydration of the tissues which a given solution produces, and also to the amount of urea, which is dissolved in the tissue at the given hydrogen ion concentration of the solution. Of course, actual injury to tissue in this method of perfusion results, and the recuperative powers must necessarily be slow, if possible at all.

In continuation of these experiments, it is proposed to test the behavior of the kidney colloids, under similar conditions, towards uric acid, creatinine, and other constituents of the normal and pathological urine.

SUMMARY AND CONCLUSION

1. The colloids of the blood serum show selective *adsorption*, holding phenolsulphonephthalein when the acidity is increased, but acting differently to glucose or urea.

2. In the excised kidney of the rabbit, perfusion of 0.9 per cent NaCl solution containing 0.5 per cent urea shows that under these conditions, and *within the physiological limits of hydrogen ion concentration,*

a. The kidney colloids secrete more urea, the less acid the perfusing solution.

b. The water secretion increases with the decrease in acidity.

c. The amount of urea retained in the kidney colloids increases with the acidity.

d. The urea excretory mechanism, while paralleling the water excretory mechanism in its operation, is quantitatively independent of it.

e. The above changes take place with very minute changes in the hydrogen ion concentration well within physiological limits.

3. The colloids of the body vary in their ability to hold dissolved substances under different degrees of hydrogen ion concentration. This suggests a basis for a mechanism of selective absorption and excretion.

4. The minute amounts of acid or alkali added (somewhere near 0.00035 gram per 100 cc.) to vary the hydrogen ion concentration, are not enough to produce the effects on a basis of osmotic differences, and we believe that we are dealing with a relationship between hydrogen ion concentrations and colloids.

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THE VAGUS NERVES IN PNEUMONIA

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I. INTRODUCTION

In earlier investigations[2] we discovered that the respiratory mechanism is exhausted in pneumonia. The normal stimulus to the bulbar respiratory cells is the carbon dioxide in the blood. To this stimulus the respiratory mechanism in pneumonia reacts imperfectly or not at all.

In pneumonia the observer is struck with the frequency and depth of the breathing. The respirations are often eighty per minute, and, in dogs, the cubic centimeters breathed per minute may rise to eighteen thousand or more. In searching for the cause of this respiratory disorder, attention was directed to the following possible explanations:

1. Such phenomena, occurring in the course of an infection, might be attributed to the action on the respiratory cells of the poison produced by the bacteria of the disease. We have accordingly measured the respiratory reaction in animals in whom the bacteremia of pneumonia was produced, without any disease of the lungs, by injecting the bacillus of Friedländer into the femoral vein.

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[2] Earlier papers in these studies of pnemonia are as follows: Arch. of Inter. Med., 1914, xiv, 48; Boston Medical and Surgical Jour., 1914, clxx, 125; *ibid.*, 1915, clxxiii, p. 472; *ibid.*, 1916, clxxiv, pp. 464–466; this Journal, 1914, xxxv, 1–14; Journ. of Exper. Med., 1915, xxii, 123; *ibid.*, 1916, xxiv, 583–603.

The first study cited consisted of measurements of the blood pressure in patients with pneumonia by Newburgh and Minot in the wards of the Massachusetts General Hospital. The remaining studies are of experimental pneumonia. These investigations, as well as the present research, were made in the Laboratory of Comparative Physiology in the Harvard Medical School, and under its direction. The cost of the present research was met in part by a grant from the Proctor Gift, contributed by the Department of Medicine in the Harvard Medical School.

2. With the idea that substances toxic to the nervous system might be formed in the pneumonic lung, the blood was withdrawn from dogs dying of pneumonia and injected into healthy dogs, whose respiratory reaction to carbon dioxide was then measured.

3. The cyanosis so common in pneumonia would suggest that a lack of oxygen might be the cause of the respiratory failure. In order to test this possibility, pneumonic animals were kept in oxygen, the partial pressure of which was many times that of the atmosphere. In other animals, oxygen was passed slowly through capillary areas.

Attempts were made to induce dyspnoea through depriving the animals of oxygen by bleeding. The effect was increased by maintaining the animals in a vertical position.

Further, the removal of oxygen was attempted by depriving the red corpuscles of their binding power by mixing illuminating gas with the inspired air.

Finally, the protection of the respiratory center from exhaustion was sought by giving prolonged artificial respiration.

4. Efforts were made to reproduce the respiratory disorder of pneumonia by placing a dog for many hours in an atmosphere containing from 7 to 12 per cent of carbon dioxide, a quantity sufficient to cause deep respirations at a rate of about fifty per minute.

5. Since the respiratory disorder in pneumonia is manifested both in the lungs and in the respiratory bulbar center, and since these two are connected by the vagus nerves, a causal relation between the dyspnoea and the exhaustion of the respiratory center was sought by section of these nerves. This procedure led us to the discovery that section of the vagus nerves protects the respiratory cells and prevents their exhaustion in pneumonia. Moreover in pneumonic dogs in which both vagus nerves have been cut, the rate of respiration remains normal.

II. GENERAL METHOD

In the experiments mentioned above the condition of the respiratory center was determined by measuring its reaction to carbon dioxide. Haldane has shown that carbon dioxide is the normal stimulus for the discharge of respiratory impulses. When an animal breathes through a closed system of tubes, as in figure 1, the carbon dioxide in the inspired air progressively increases. In normal animals, the increase in the carbon dioxide stimulates the respiratory center and causes a corresponding increase in the volume of the air passing in and out of

the chest. Thus, in cats and dogs, when the carbon dioxide rises to 3 per cent, the volume of the air inspired is usually doubled. The condition of the respiratory mechanism is revealed by the volume of air breathed per minute as the carbon dioxide in the inspired air rises from 1 to 5 per cent.

The apparatus for this measurement is shown in figure 1. The tracheal cannula of the animal in which the respiratory reaction was to be measured was joined to a rubber tube placed between two Tissot valves[3] connected in such a way that the animal breathed into a spirometer[4] and out of a bottle connected in its turn with the spirometer, so that the lungs, the spirometer, the bottle, and the connecting tubes formed a closed system. Evidently, by this method the volume

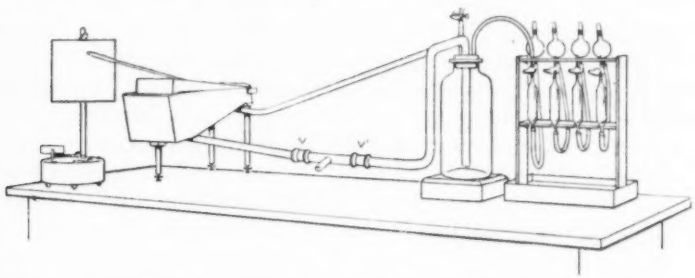

Fig. 1. Apparatus for measuring the respiratory reaction to carbon dioxide. From left to right; the kymograph; the spirometer; the Tissot valves, v,v^1; the bottle; the mercury sample tubes. The electromagnetic signal and the Haldane Apparatus are not shown.

of the air passing into and out of the chest was recorded by the spirometer, while the carbon dioxide exhaled by the animal constantly accumulated in the closed system.

Samples of the air thus enriched with the carbon dioxide were withdrawn at frequent intervals by the mercury tubes shown in figure 1. As each sample was taken, a mark was made on the spirometer record above the time record (the electric time signal is not shown in fig. 1). As the carbon dioxide in the respired air increased, the spirometer curve became deeper until the maximum reaction was reached.[5] The spirometer was empirically graduated by recording the vertical rise of its

[3] Metal flap valves so lightly constructed as not to impede respiration.

[4] This aeroplethysmograph was invented by Prof. J. Gad.

[5] Shown in figure 3.

writing point with each increase of 50 cc. in its contents. When the experiment was finished the empirical graduation scale was used to measure the number of cubic centimeters breathed in the minute nearest the mark at which the air sample was withdrawn. The air in the sample was analyzed for carbon dioxide with a Haldane apparatus and the percentage written against the ventilation (see table 1). Thus, the cubic centimeters breathed per minute as the carbon dioxide rose from the atmospheric level to 3 per cent or more were recorded accurately. When plotted on coördinate paper[6] a curve results, expressing the reaction of the respiratory mechanism to increasing quantities of carbon dioxide. Upon this curve may be read the cubic centimeters breathed per minute at 1, 2, 3, or 4 per cent of carbon dioxide. The data for tables 1 to 4 were obtained in this way.

The organism employed was the *Bacillus* pneumoniae of Friedländer.[7] It was passed through three guinea pigs to increase its virulence to such a degree that 1 cc. of a broth culture injected into the peritoneum killed a guinea pig in twelve hours. Its virulence was kept at this point by occasional passage through additional guinea pigs.

Pneumonia was produced by injecting into the trachea, broth cultures incubated from eighteen to twenty-four hours. The quantity injected varied; we found that 2 cc. per kilo caused death in dogs usually in about thirty hours.

In pneumonic dogs with intact vagi, the fatal issue is usually preceded by a characteristic fall in temperature; this fall was often absent in pneumonic dogs in which the vigi had been cut.

The measurements of pneumonic animals made in this investigation were made only on cats and dogs that afterwards died of the disease, and in whom an autopsy showed the presence of typical consolidation.

All operations that might give pain were performed under ether or morphine or both.[8]

III. PRELIMINARY EXPERIMENTS

The injection of the Bacillus pneumoniae into a vein. In order to separate the possible action of the bacterial poison upon the bulbar cells from the pneumonic process in the lung, a lethal dose of a culture of the Friedländer bacillus was injected into a vein.

[6] Shown in figure 4.

[7] For the bacteriological examination we are indebted to Dr. C. C Page. See Newburgh, Means and Porter, the Journ. of Exper. Med., 1916, xxiv, 583–603.

[8] For further details regarding method, see Newburgh, Means and Porter, loc. cit.

In the experiment of February 2, 1916, 15 cc. of a broth culture were injected into the femoral vein of a dog at 4 p.m. The rectal temperature rose to 40°C. An hour before death, the temperature had fallen to 38°. The respiratory reaction was measured at six hours and at one hour before death, which occurred thirty hours after inoculation. The second measurement is given in table 1. It was made while the animal was in complete coma, and wholly insensitive to pain. The respiratory reaction is normal. A second experiment also gave a normal result.

TABLE 1

The respiratory reaction to carbon dioxide in a dog in which Bacillus pneumoniae was injected into the femoral vein, causing a fatal bacteremia without injury to the lungs. One hour before death.

PERCENT OF CARBON DIOXIDE IN THE INSPIRED AIR	RESPIRATIONS PER MINUTE	AVERAGE VOLUME OF ONE RESPIRATION	TIDAL AIR PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR
per cent		*cc.*	*cc.*	*per cent*
Room air	16.4	175	2,870	
1.87	17.0	220	3,740	30
3.46	20.4	330	6,730	134
5.16	28.5	460	12,650	340
6.17	54.0	470	25,400	785

These experiments are evidence that the failure of the respiratory center in pneumonia cannot be explained by the bacterial poison in the blood.

The injection of pneumonic blood into healthy dogs. It is conceivable that the pathological process in the lung might develop poisons which, when passed into the blood, would reach the respiratory nerve cells. Evidence against this possibility was obtained by injecting into a healthy dog, previously bled, the blood from dogs about to die from pneumonia. The protocol of this experiment follows:

Experiment on dog 39, March 1, 1915

February 28, 12.30 p.m., dog 37, weight 13.5 kilos, was injected in the trachea with 28 cc. of a broth culture of Bacillus pneumoniae. A second dog, no. 38, weight 17 kilos, received 17 cc. of the culture.

February 29, 7.30 p.m. Dog 37 cannot stand. Temperature 40°C. He is bled 250 cc. from the carotid artery.

8.30 p.m. This blood is introduced into the femoral vein of a normal dog (no. 39), whose respiratory reaction is then tested.

March 1, 1.45 a.m. Dog 38 is in coma. Temperature is falling. He is bled from the carotid artery. The artery is nearly empty. The blood is dark and

thick. 400 cc. of this blood is injected into dog 39 and several hours afterward the respiratory reaction of no. 39 is again measured. It was normal. On room air he breathed 2970 cc. per minute; when the inspired air contained 3 per cent of carbon dioxide, the increase in tidal air was 87 per cent; and at 5 per cent it was 310 per cent.

This experiment gives no support to the hypothesis that the bulbar cells are poisoned through the blood by products arising in the pneumonic lung.

The influence of oxygen and of prolonged artificial respiration. One of the common clinical signs in pneumonia is the early and prolonged cyanosis.

This would suggest that the respiratory disorder could be modified by increasing the available oxygen. Not being certain that the red corpuscles were in a condition to bind their normal quantity of oxygen, it was resolved to place the pneumonic animal in oxygen the partial pressure of which should be great enough to enable large quantities to enter the plasma, thus rendering the respiratory function of the corpuscles more or less superfluous. Pneumonic cats and dogs were placed in a galvanized iron tank provided with a flanged opening. Against this opening, a flanged door, containing a glass window, was firmly bolted. An oxygen cylinder with a reducing valve was connected to a tube leading into the respiration tank and an outflow tube was joined to a safety valve. The animals were surrounded for many hours by oxygen at a pressure of 25 pounds per square inch. The gas within the tank was slowly renewed as it gradually escaped through the outlet valve. A dish of sodium hydrate absorbed some, at least, of the carbon dioxide. As the course of the disease did not seem to be modified by this immersion in oxygen, the experiments were repeated under conditions made more favorable by the continuous removal of the carbon dioxide produced by the animal's respiration. As the apparatus for this purpose may be of interest, a diagram is supplied (fig. 2). A motor drives a pump which forces oxygen through a recording gauge into a galvanized iron tank containing the animal. From the tank the oxygen passes by an outlet valve through sulphuric acid and soda lime into a T tube. One limb of this T conducts the gas to the intake of the pump; the other limb draws from a spirometer fed through a reducing valve from a cylinder containing oxygen under pressure. The outlet valve of the animal tank was almost closed so that the gases passed through the valve at a pressure just sufficient to drive them through the sulphuric acid and soda lime to the intake of the pump.

During this passage the gases were dried and the carbon dioxide removed. Frequent analyses showed that the carbon dioxide in the animal tank was less than 0.5 per cent. The oxygen abstracted by the animal was replaced from the oxygen cylinder by an assistant who allowed gas to pass through the reducing valve at a rate that would keep the spirometer cover from either rising or falling.

In none of the numerous experiments in which the animals were kept for hours in oxygen at a high partial pressure was there any clear evidence that the progress of the disease was checked or the condition of the animal materially improved. It was particularly noticeable that no significant change occurred either in the frequency or the depth of the respiration.

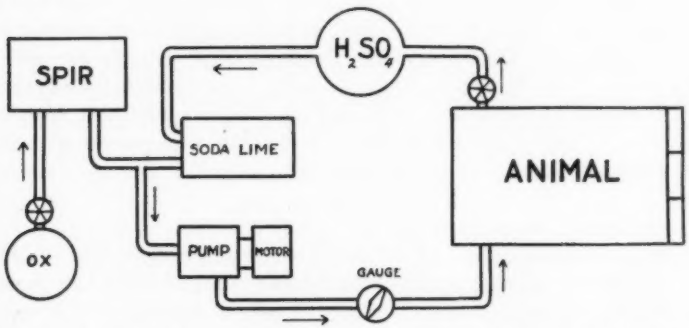

Fig. 2. Apparatus for maintaining an animal under pressure and free from carbon dioxide.

Not having produced a favorable result by immersing the animal in oxygen under pressure, the forlorn hope remained that pure oxygen could be passed through a capillary area so slowly that it might be taken up by the red corpuscles quickly enough to avoid gas embolism. Oxygen was therefore allowed to enter very slowly the peripheral end of the femoral artery, and the femoral vein was exposed to observe the result. The outcome was interesting. A succession of small cylinders of deeply venous blood passed through the vein, each separated from the next by an approximately equal mass of oxygen. No change in the color of the venous blood was observed, even where it was in contact with the gas. After a few minutes, the gas collected in the heart and a rough hissing sound could be distinctly heard with each contraction. Death followed very shortly.

In the attempt to secure evidence that the dyspnoea was due to lack of oxygen, large numbers of red corpuscles were withdrawn by bleeding and the possible anaemia of the bulbar cells was further increased by placing the animals in a vertical position. These experiments failed to produce dyspnoea.

Efforts were also made to produce oxygen hunger by uniting the haemoglobin with carbon monoxide. To this end, illuminating gas was mixed with the inspired air. Dyspnoea did not appear, even when the carbon monoxide was pushed so far that the dogs became comatose.

Since the prolonged dyspnoea in pneumonia must obviously be exhausting, attempts were made to rest the respiratory apparatus by artificial respiration and thus conserve the animal's strength until the crisis might give a favorable turn to the disease. This hope was not realized. The animals with intact vagi appeared to resist the artificial respiration; the frequency of their respiratory discharges did not diminish. Dogs in which section of both vagus nerves caused the irregular spasmodic type of vagal breathing sometimes seemed more comfortable under artificial respiration, and their temperature sometimes fell several degrees, but death commonly followed after the usual interval.

Hyperpnoea from inhalation of carbon dioxide. Trusting that the respiratory center would be exhausted by rapid, deep respirations, extending over many hours, a dog was placed in an air-tight box through which was passed a stream of air containing carbon dioxide and oxygen. The experiment began at 2.30 p.m., February 15, 1916. During nine hours, the dog breathed an atmosphere containing from 7.58 to 12.40 per cent of carbon dioxide. The oxygen varied from 16.6 to 21.8 per cent. The respirations averaged about 50 per minute.

At 11.30 p.m. the dog was taken out of the carbon dioxide box and his respiratory reaction was immediately tested. On breathing room air, the tidal flow was 2800 cc. per minute; on breathing air containing 6.94 per cent carbon dioxide, the ventilation reached the extraordinary figure of 28,200 cc. per minute, an increase of 900 per cent. The respiratory center had not been impaired by the prolonged hyperpnoea.

It will be seen from the preceding account that the number of experiments performed to test each hypothesis was small. Nevertheless, the experiments sufficiently indicated that none of these hypotheses explained the exhaustion of the respiratory apparatus in pneumonia.

Attention was therefore directed toward a fifth possibility, namely, that during the disease, nerve impulses arising in the lung are trans-

mitted along the vagus nerves to the bulbar respiratory cells. These vagal impulses, acting through many hours, might modify the condition of the respiratory cells in such a way that they could no longer react to carbon dioxide. This hypothesis derives weight from the striking difference in the results of injecting a fatal dose of the Bacillus pneumoniae into a vein as compared with those which follow its injection into the lungs. In both cases there is a bacteraemia. When this bacteraemia is associated with inflammation of the lungs, a panting respiration is the most prominent symptom of the disease. When the lungs are not affected, the breathing remains normal until the animal dies. The lungs can reach the bulbar respiratory cells through the blood or through the vagus nerves. The changes in the blood do not produce dyspnoea. We therefore turned to the vagus nerves. The experiments in support of the vagal hypothesis are divided into two groups: first, those in which both vagus nerves were cut in the neck; second, those in which the left vagus was cut in the neck, and the right vagus was cut in the chest after the manner of Pawlow.[9]

IV. THE RESPIRATORY REACTION IN PNEUMONIC DOGS WITH BOTH VAGI CUT IN THE NECK

Table 2 presents the respiratory reaction to carbon dioxide in pneumonic dogs in which both vagus nerves were cut on a level with the upper rings of the trachea. It will be noted that these measurements were made from two and three-quarters hours to fifteen minutes before death. The average reaction in normal dogs on inspiration of air containing 3 per cent of carbon dioxide is an increase of 96 per cent in the tidal air per minute. In the six vagotomized pneumonic dogs, the reaction is 84 per cent. One dog increased 142 per cent. Dog 45 gave a very poor reaction. This was because we were unable to get him to breathe quietly at the beginning of the test. If this experiment be omitted, the remaining five, when the inspired air contained 3 per cent of carbon dioxide, will show an increase of 91 per cent in the tidal air. The reaction in these vagotomized pneumonic dogs was normal. On the other hand, dogs at the same stage of the disease but with vagi intact increased their tidal air by only 46 per cent. Hence, section of the vagus nerves protects the respiratory mechanism from the impairment always observed in this disease when the vagus nerves are intact.

[9] Pawlow, J. P.: The Work of the Digestive Glands (transl. by W. H. Thompson), Chas. Griffin & Co., 1910. 2nd English Ed. 54.

TABLE 2

Respiratory reaction to carbon dioxide by pneumonic dogs with both vagi cut in the neck during the course of the disease

NUMBER OF DOG	EXPERIMENT	WEIGHT	DATE 1916	HOURS BEFORE DEATH	RECTAL TEMPERATURE	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION RATE PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR AS CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3 PER CENT		
								1	2	3
		kilos				*cc.*		*per cent*	*per cent*	*per cent*
44	B	5.5	March 11	¾	35°	2,270	13	14	45	93
45	A	8.0	March 12	½	40°	4,640	18	17	32	45
51	B	4.5	March 14	2	31°	1,500	8	24	57	88
54	A	8.5	March 26	½	40°	3,300	20	18	42	64
56	A	6.5	March 25	2¾	36.5°	3,040	13	11	42	70
57	B	6.5	March 26	¼	39.5°	2,520	21	48	116	142
Average.....		6.6				2,878	16	22	56	84

The reaction to carbon dioxide in the pneumonic vagotomized dog recorded in table 2 is indeed greater than the reaction observed in three vagotomized dogs without pneumonia. The measurements of these normal vagotomized dogs are given in table 3. At 3 per cent of carbon dioxide they increased their tidal air by 68 per cent.

It will be observed in table 2 that the initial ventilation averages 2878 cc. per minute. This is not unusual in dogs, the average weight of which is but 6.6 kilos. Thus in an experiment performed April 13, 1916, a dog wholly normal, except for tracheotomy, had an initial ventilation of 1610 cc. per minute. When the air inspired contained 3

TABLE 3

Respiratory reaction to carbon dioxide in normal dogs with both vagi cut in the neck

NUMBER OF DOG	EXPERIMENT	WEIGHT	DATE 1916	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR WHEN THE CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3.5 PER CENT			
						1	2	3	3.5
		kilos		*cc.*		*per cent*	*per cent*	*per cent*	*per cent*
58	A	8.0	March 29	3,182	25	23	44	66	90
66	A	5.5	April 7	1,870	8	20	42	79	104
71	C	4.5	April 14	1,460	12	21	41	60	85
Average.....				2,171	15	21	42	68	93

per cent of carbon dioxide, his ventilation increased 118 per cent. His weight was 4.5 kilos.

The reader is reminded that the decrease in rate after double vagus section is usually held to be balanced by an increase in depth, so that the total ventilation remains substantially the same. Without making any general statement with regard to this, it may be pointed out that the initial ventilation in two of the three vagotomized but otherwise normal dogs listed in table 3 was greater than the ventilation in the wholly normal dog of April 13.

The reader will also remember that Haldane has conclusively shown that ventilation is not to be measured simply by the tidal air. A difference between the air that passes in and out of the chest and the air that passes in and out of the alveoli must be kept in mind. Deep infrequent breathing may ventilate the alveoli better than frequent shallow breathing, even when the shallow breathing brings more air per minute into the chest. In shallow breathing, a greater portion of the tidal air may be spent on the dead space. In short, it does not follow that a small ventilation in small vagotomized dogs indicates abnormally small tidal movement or is necessarily an evidence of imperfect ventilation.

If the five vagotomized dogs in table 2 whose initial ventilation is small reacted vigorously to carbon dioxide because of their small initial ventilation, a vagotomized dog whose initial ventilation is large should react less vigorously. In our experience this is not the case. On June 13, the respiratory reaction was measured in a vagotomized dog (no. 91) fifty minutes before death from pneumonia. The initial ventilation was 5140 cc. per minute. When the inspired air contained 3 per cent of carbon dioxide, the ventilation was doubled. On June 28, a vagotomized dog (no. 100) was tested just before death from pneumonia. His initial ventilation was 5300 cc. per minute. At 3 per cent of carbon dioxide, the ventilation was 10,000 cc. per minute.

When the tidal air per minute is divided by the weight of the animal in kilos, a ventilation which appears small may be found normal. Thus the average ventilation of the six vagotomized pneumonic dogs in table 2 is 2878 cc. per minute. The dog of June 13 had an initial ventilation 5140 cc. per minute. But the dogs of table 2 weighed as an average 6.6 kilos and the dog of June 13 weighed 12 kilos. The ventilation per minute per kilo in the dogs of table 2 was 437 cc., while in the dog of June 13, which weighed twice as much, it was 442 cc. If this dog of June 13, which weighed 12 kilos and breathed 442 cc. per minute

per kilo is compared with the vagotomized pneumonic dog 56 (table 2), who weighed one-half as much but who breathed 468 cc. per minute per kilo, it is at once clear that the small initial ventilation of the vagotomized dogs of table 2 is apparent rather than real.

In our communication on "The Respiratory Mechanism in Pneumonia," we showed at length that the reaction to carbon dioxide was independent of the initial ventilation. It is not necessary to go over this ground again.[10]

It will hardly be denied that a critical examination of table 2 bears out the assertion that double vagotomy prevents the exhaustion of the respiratory center.

V. THE RESPIRATORY REACTION IN PNEUMONIC DOGS WITH ONE VAGUS CUT IN THE CHEST AND THE OTHER VAGUS CUT IN THE NECK

For our purposes, the section of both vagus nerves in the neck has serious disadvantages. If the nerves were cut during the course of the disease, the dogs would sometimes become markedly cyanotic and would then struggle, apparently for oxygen. The breathing in these cases was usually spasmodic and irregular. Such animals are obviously unsuited for testing the respiratory reaction. None such were used in table 2, though one restless dog (no. 45) was accepted with the reservation already noted. Moreover, death often took place before the period at which it would have been expected had not the vagus nerves been cut. As it was important for our results to test the respiratory reaction just before the death from pneumonia, premature death from an intercurrent operation could not be passed over. To avoid this difficulty, we planned to cut the vagus nerves and to allow the animals to recover before they were inoculated. This proved to be impracticable. After double vagotomy in the neck, the dogs could retain neither food nor drink. They all died within three days. It seems that a similar obstacle was encountered by Pawlow,[11] who overcame it by severing the right vagus in the chest, between the cardiac and the pulmonary branches. This point is considerably distal to the origin of the recurrent laryngeal nerve. As the procedure has been highly useful, a description of our technique may not be out of place.

A strong, short-haired dog[12] weighing about 8 kilos is tied upon a well scrubbed board and etherized. When the anaesthesia is sufficiently

[10] See The Journal of Experimental Medicine, 1916, xxiv, 583–603.

[11] Pawlow, J. P. loc. cit.

[12] Dogs with distemper should be carefully avoided.

deep, the mouth is held open by straps passing behind the canine teeth, the tongue is drawn forward and a rubber tube almost the size of the trachea is passed through the glottis down to the bifurcation. At the other end of the tube is a glass Y, one limb of which is left open, to be stopped at will by the finger of the anaesthetist. The third limb is connected with an ether flask through which passes a continuous air current. An additional inhalation of ether can be secured by standing the ether flask in a basin of warm water. The hair is now closely cut over the right upper chest, which is then thoroughly scrubbed with a broad nail brush and hot soapsuds. This region, except the second intercostal space, is then covered with clean towels soaked in corrosive sublimate solution. The instruments are two large, broad retractors, one long and one short dissecting forceps, curved and straight scissors, a right-angled artery hook, a "seeker," six long-handled haemostatic forceps, eight straight and four curved threaded needles, several ligatures, a knife, and a dozen "sponges" of absorbent cotton wrung out of corrosive sublimate solution and placed in a sterile beaker. The instruments were thoroughly boiled before each operation, and were then laid out upon a small movable stand which had been covered with a sterile cloth. The operator, the assistant, and the anaesthetist wore the usual white suits. The operator used a frontal mirror to light the interior of the chest. An incision was made through the skin and the muscles over the second intercostal space from the border of the axilla to the edge of the sternum. The chest was opened in this line and the opening widened with two retractors, held by the assistant. Care must be taken not to press on the superior vena cava. The right vagus nerve lies on the trachea beneath the superior vena cava. The connective tissue is grasped with the long dissecting forceps, the nerve is freed with the seeker, held gently with the forceps, and severed on both sides of that instrument. The removal of a portion of the nerve forestalls any doubt as to its section. The right vagus nerve lies beneath the superior vena cava; the right phrenic nerve lies upon that vein and must on no account be injured. With practice, the vagus operation can be done in a few minutes. When the retractors are withdrawn, the divided pectoralis major muscle is brought together with three or four deep ligatures placed with curved needles. The blades of a forceps are now passed into the chest between two of the deep ligatures and the anaesthetist, by stopping the free end of the Y tube with his finger, causes the lung to expand and drive the air out of the chest. At this moment the forceps is rapidly withdrawn and the skin wound closed

with ligatures placed about 1 cm. apart. No dressing is used. Throughout the operation, the dog must be kept anaesthetized by stopping the free end of the Y tube sufficiently often to reproduce the natural respiratory movements of the lung. This intratracheal anaesthesia avoids a tracheotomy, the healing of which is very difficult.

The operation can be warmly recommended. We rarely failed to secure a good recovery within a few days. From three days to a week after the first operation, a small incision was made in the neck over the left vagus nerve, the carotid sheath with the nerve brought to the surface with a curved blunt instrument, and the nerve freed and severed.

Twenty-four hours after the double vagotomy had been completed, the dog ate and drank with comfort. The breathing was vagal in type, but was so easy that the increased depth and the diminished rate

TABLE 4

Respiratory reaction to carbon dioxide in pneumonic dogs with one vagus cut in the neck and one cut in the chest previous to the disease

NUMBER OF DOG	EXPERIMENT	WEIGHT	DATE 1916	HOURS BEFORE DEATH	TEMPERATURE	INITIAL VENTILATION PER MINUTE	INITIAL RESPIRATION PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR AS THE CARBON DIOXIDE IN THE INSPIRED AIR INCREASED FROM 1 TO 3 PER CENT		
								1	2	3
		kilos				cc.		*per cent*	*per cent*	*per cent*
91	C	10.5	June 13	50 min.	102°	5,140	21	20	53	102
100	C	12.0	June 28	2 min.		5,100	12.5	28	57	88
Average.....		11.3				5,120	17	24	55	95

might readily escape notice. Indeed, the vagal type could not be made out unless the animal were resting quietly.

These animals were usually inoculated with pneumonia from three to seven days after the second vagus nerve was cut. The disease ran its usual course except that the characteristic final fall in temperature was frequently absent and the sudden death was not infrequent. Some animals were lost because they died before partial coma had set in, thus preventing the testing of the respiratory reaction. The animal must be quiet throughout this test.[13] Ether is of course impossible. Decerebration cannot be used with double vagotomy because this conjunction produces long, irregular respiratory spasms. It is therefore necessary to wait until the animal is at least partially comatose.

[13] The test has often been made on men and women. It is quite painless.

In pneumonic dogs with vagi intact the hour of death can be approximately calculated, but in pneumonic dogs with both vagi cut this calculation is much more difficult. The observer who may repeat these experiments will do well to remain continuously with his animals, as we have done, during the thirty or forty hours of the disease.

The respiratory reaction to carbon dioxide in pneumonic dogs with both vagi cut in the manner described above is shown in table 4. The reaction of dog 91, fifty minutes before death, is shown in figures 3 and 4. The protocol of this experiment follows:

Experiment June 2, 1916. Dog No. 91, weight 10.5 kilos

June 2, 11 p.m. Right vagus cut inside the chest.

June 7, 4.10 p.m. Left vagus cut in the neck.

June 12, 4.15 p.m. Bronchus injected with 3 cc. per kilo broth culture of Bacillus pneumoniae.

June 13, 9.15 a.m. Temperature 105°, respiration 18.

June 13, 11 a.m. Temperature 104.8°. Carbon dioxide reaction measured (A).

June 13, 11.30 a.m. Temperature 101.5. Second measurement (B).

June 13, 12.30 p.m. Temperature 101.3. Coma. Third measurement (C) of carbon dioxide reaction.

June 13, 1.20 p.m. Death.

Autopsy. Both vagus nerves severed. Red hepatization of three-fourths of right lower and one-half of right middle lobes.

An examination of table 4 and figures 3 and 4 shows that the respiratory reaction of these vagotomized pneumonic dogs is normal, the average increase in tidal air is 95 per cent, when the inspired air contains 3 per cent of carbon dioxide. In pneumonic dogs with intact vagi, this reaction averaged 46 per cent. In normal dogs, it averaged 96 per cent. The dogs in table 4 had a fairly large initial ventilation, averaging 5120 cc. per minute, or 453 cc. per minute per kilo. The ventilation of five normal dogs averaged 4344 cc. per minute. As in table 2, the dogs with double vagotomy have no dyspnoea in spite of a disease in which dyspnoea is, with intact vagi, the most prominent symptom.

A critical examination of the experiments in table 4 would naturally consider the duration of the disease. The average duration of life in pneumonic dogs with intact vagi, is thirty hours. The six dogs of table 2, in whom both vagi were cut in the neck during the course of the disease, also lived, as an average, thirty hours. Section of both vagi in this manner and at this time does not therefore shorten the duration of the pneumonia. On the other hand, ten dogs in whom, previous to

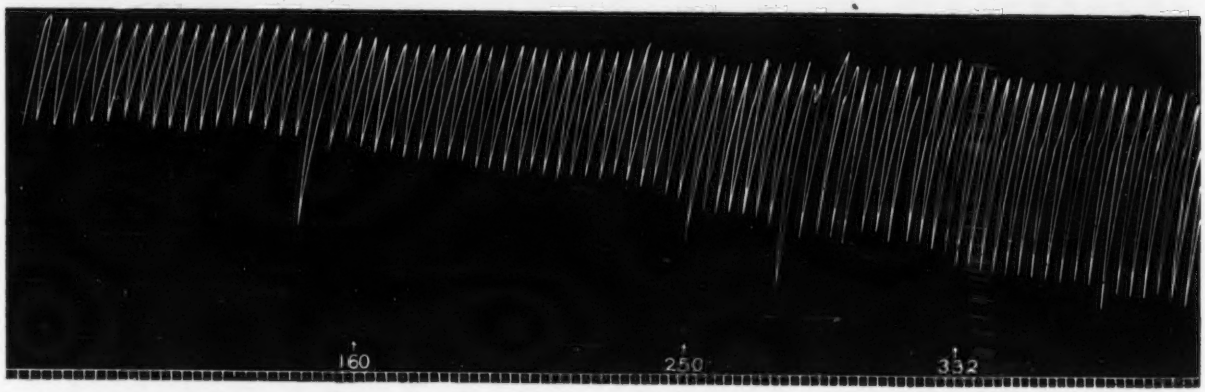

Fig. 3. Normal respiratory reaction of the vagotomized dog, no. 91, June 13, 1916, fifty minutes before death from pneumonia. In room air, this dog breathed 5140 cc. per minute. In air containing 3 per cent of carbon dioxide, the tidal air increased 102 per cent.

inoculation, one vagus was cut in the chest and the other in the neck, lived, as an average, but twenty hours after inoculation. It might, therefore, be said that the dogs in table 4 gave a normal reaction because they were tested ten hours before the pneumonia alone would have killed them. Out of this criticism two questions arise: First, does pneumonia ever kill dogs with intact vagi in twenty hours? Second, if there are such cases what respiratory reaction do they give shortly before death; or, in other words, what is the result of measuring their reaction at the same period in the disease at which the reaction of the dogs in table 4 was measured? These questions are answered by the

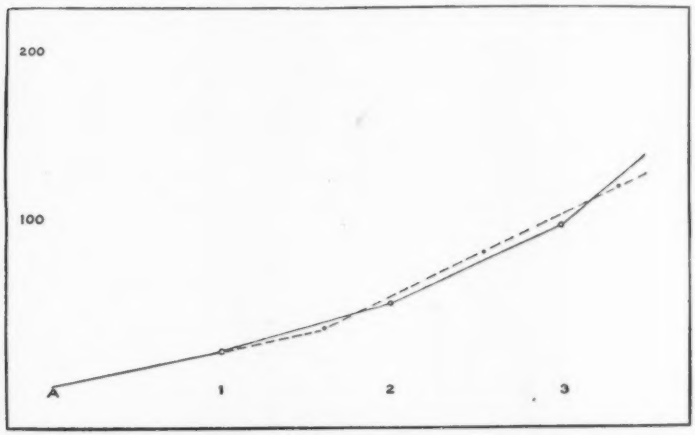

Fig. 4. The broken line is the respiratory reaction of a vagotomized dog (no. 91) fifty minutes before death from pneumonia. The unbroken line is the average reaction of five normal dogs.

experiments of November 26 and November 28, 1915. In the first of these experiments, dog no. 6 lived twenty-three hours and his respiratory reaction at 3 per cent of carbon dioxide was 60 per cent measured three hours before death. His temperature was 35°C. This same dog two hours previously gave a normal reaction but his temperature at that time was 38°C. It had probably been higher but since it fell finally to 33° there is some reason to believe that the fatal fall had just begun at the time when this perfect reaction was obtained. The second dog observed on November 28 lived eighteen hours. His respiratory reaction was taken one hour before death when the temperature

was 34° and the usual coma was present. When the carbon dioxide in the inspired air was 3 per cent his tidal air was increased 64 per cent. These facts justify the following statement. Out of five pneumonic dogs with intact vagi, two lived no longer than the dogs in table 4. When the inspired air contained 3 per cent of carbon dioxide these two increased their tidal air by 62 per cent, whereas the dogs in table 4 measured at the same stage of the disease increased 95 per cent.

Additional strength is imparted to table 4 by the history of the third dog of the group of five just mentioned. This animal, no. 14, gave on December 10, 1915, an increase of only 12 per cent in the tidal air when the inspired air contained 3 per cent of carbon dioxide, twenty-four hours after inoculation.

VI. CONCLUSIONS

1. Section of both vagus nerves prevents the exhaustion of the respiratory mechanism in experimental pneumonia.

2. In such animals there is no dyspnoea. The rate of respiration remains practically unchanged throughout the disease.